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PROSTATE-SPECIFIC MEMBRANE ANTIGEN

Background of the Invention

This application is a continuation-in-part of United States Application Serial No. 07/973,337, filed on November 5, 1992, the contents of which are hereby incorporated by reference.

This invention disclosed herein was made in part with Government support under NIH Grants No. DK47650 and CA58192 from the Department of Health and HUman Services. Accordingly, the U.S. Government has certain rights in this invention.

Throughout this application various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of of each series of experiments.

Prostate cancer is among the most significant medical problems in the United States, as the disease is now the most common malignancy diagnosed in American males. In 1992 there were over 132,000 new cases of prostate cancer detected with over 36,000 deaths attributable to the disease, representing a 17.3% increase over 4 years (2). Five year survival rates for patients with prostate cancer range from 88% for those with localized disease to 29% for those with metastatic disease. The rapid increase

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in the number of cases appears to result in part from an
increase in disease awareness as well as the widespread
use of clinical markers such as the secreted proteins
prostate-specific antigen (PSA) and prostatic acid
5   phosphatase (PAP) (37).

The prostate gland is a site of significant pathology
affected by conditions such as benign growth (BPH),
neoplasia (prostatic cancer) and infection (prostatitis).
10   Prostate cancer represents the second leading cause of
death from cancer in man (1). However prostatic cancer
is the leading site for cancer development in men. The
difference between these two facts relates to prostatic
cancer occurring with increasing frequency as men age,
15   especially in the ages beyond 60 at a time when death
from other factors often intervenes. Also, the spectrum
of biologic aggressiveness of prostatic cancer is great,
so that in some men following detection the tumor remains
a latent histologic tumor and does not become clinically
20   significant, whereas in other it progresses rapidly,
metastasizes and kills the man in a relatively short 2-5
year period (1, 3).

In prostate cancer cells, two specific proteins that are
25   made in very high concentrations are prostatic acid
phosphatase (PAP) and prostate specific antigen (PSA) (4,
5, 6). These proteins have been characterized and have
been used to follow response to therapy. With the
development of cancer, the normal architecture of the
30   gland becomes altered, including loss of the normal duct
structure for the removal of secretions and thus the
secretions reach the serum. Indeed measurement of serum
PSA is suggested as a potential screening method for
prostatic cancer. Indeed, the relative amount of PSA
35   and/or PAP in the cancer reduces as compared to normal or

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benign tissue.

PAP was one of the earliest serum markers for detecting
metastatic spread (4). PAP hydrolyses tyrosine phosphate
5   and has a broad substrate specificity. Tyrosine
phosphorylation is often increased with oncogenic
transformation. It has been hypothesized that during
neoplastic transformation there is less phosphatase
activity available to inactivate proteins that are
10   activated by phosphorylation on tyrosine residues. In
some instances, insertion of phosphatases that have
tyrosine phosphatase activity has reversed the malignant
phenotype.

15   PSA is a protease and it is not readily appreciated how
loss of its activity correlates with cancer development
(5, 6). The proteolytic activity of PSA is inhibited by
zinc. Zinc concentrations are high in the normal
prostate and reduced in prostatic cancer. Possibly the
20   loss of zinc allows for increased proteolytic activity by
PSA. As proteases are involved in metastasis and some
proteases stimulate mitotic activity, the potentially
increased activity of PSA could be hypothesized to play
a role in the tumors metastases and spread (7).

25

Both PSA and PAP are found in prostatic secretions. Both
appear to be dependent on the presence of androgens for
their production and are substantially reduced following
androgen deprivation.

30

Prostate-specific membrane antigen (PSM) which appears to
be localized to the prostatic membrane has been
identified. This antigen was identified as the result of
generating monoclonal antibodies to a prostatic cancer
35   cell, LNCaP (8).

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Dr. Horoszewicz established a cell line designated LNCaP from the lymph node of a hormone refractory, heavily pretreated patient (9). This line was found to have an aneuploid human male karyotype. It maintained prostatic differentiation functionality in that it produced both PSA and PAP. It possessed an androgen receptor of high affinity and specificity. Mice were immunized with LNCaP cells and hybridomas were derived from sensitized animals. A monoclonal antibody was derived and was designated 7E11-C5 (8). The antibody staining was consistent with a membrane location and isolated fractions of LNCaP cell membranes exhibited a strongly positive reaction with immunoblotting and ELISA techniques. This antibody did not inhibit or enhance the growth of LNCaP cells in vitro or in vivo. The antibody to this antigen was remarkably specific to prostatic epithelial cells, as no reactivity was observed in any other component. Immunohistochemical staining of cancerous epithelial cells was more intense than that of normal or benign epithelial cells.

Dr. Horoszewicz also reported detection of immunoreactive material using 7E11-C5 in serum of prostatic cancer patients (8). The immunoreactivity was detectable in nearly 60% of patients with stage D-2 disease and in a slightly lower percentage of patients with earlier stage disease, but the numbers of patients in the latter group are small. Patients with benign prostatic hyperplasia (BPH) were negative. Patients with no apparent disease were negative, but 50-60% of patients in remission yet with active stable disease or with progression demonstrated positive serum reactivity. Patients with non prostatic tumors did not show immunoreactivity with 7E11-C5.

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The 7E11-C5 monoclonal antibody is currently in clinical trials. The aldehyde groups of the antibody were oxidized and the linker-chelator glycol-tyrosyl- (n, ϵ-diethylenetriamine-pentacetic acid)-lysine (GYK-DTPA) was
5   coupled to the reactive aldehydes of the heavy chain (10). The resulting antibody was designated CYT-356. Immunohistochemical staining patterns were similar except that the CYT-356 modified antibody stained skeletal muscle. The comparison of CYT-356 with 7E11-C5
10 monoclonal antibody suggested both had binding to type 2 muscle fibers. The reason for the discrepancy with the earlier study, which reported skeletal muscle to be negative, was suggested to be due to differences in tissue fixation techniques. Still, the most intense and
15 definite reaction was observed with prostatic epithelial cells, especially cancerous cells. Reactivity with mouse skeletal muscle was detected with immunohistochemistry but not in imaging studies. The Indium[111]-labeled antibody localized to LNCaP tumors grown in nude mice with an
20 uptake of nearly 30% of the injected dose per gram tumor at four days. In-vivo, no selective retention of the antibody was observed in antigen negative tumors such as PC-3 and DU-145, or by skeletal muscle.

25 Very little was known about the PSM antigen. An effort at purification and characterization has been described at meetings by Dr. George Wright and colleagues (11, 12). These investigators have shown that following electrophoresis on acrylamide gels and Western blotting,
30 the PSM antigen maintains a molecular weight of 100 kilodaltons (kd). Chemical and enzymatic treatment showed that both the peptide and carbohydrate moieties of the PSM antigen are required for recognition by the 7E11-C5 monoclonal antibody. Competitive binding studies with

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specific lectins suggested that galNAc is the dominant carbohydrate of the antigenic epitope.

5   The 100kd glycoprotein unique to prostate cells and tissues was purified and characterized. The protein was digested proteolytically with trypsin and nine peptide fragments were sequenced. Using the technique of degenerate PCR (polymerase chain reaction), the full-length 2.65 kilobase (kb) cDNA coding for this antigen

10   was cloned. Preliminary results have revealed that this antigen is highly expressed in prostate cancer tissues, including bone and lymph node metastases (13). The entire DNA sequence for the cDNA as well as the predicted amino acid sequence for the antigen was determined.

15   Further characterization of the PSM antigen is presently underway in the applicants' laboratory including: analysis of PSM gene expression in a wide variety of tissues, transfection of the PSM gene into cells not expressing the antigen, chromosome localization of the

20   PSM gene, cloning of the genomic PSM gene with analysis of the PSM promoter and generation of polyclonal and monoclonal antibodies against highly antigenic peptide domains of the PSM antigen, and identification of any endogenous PSM binding molecules (ligands).

25

Currently, LNCaP cells provide the best in-vitro model system to study human prostate cancer, since they produce all three prostatic bio-markers; PSA, PAP and PSM. The cells possess an aneuploid male karyotype with a Y

30   chromosome, express a high affinity androgen receptor, and are hormonally responsive to both testosterone and DHT. Because PSM appears to be a transmembrane glycoprotein, it is considered an attractive target for both antibody-directed imaging and targeting of prostatic

35   tumor deposits (38). We have demonstrated expression of

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PSM protein in LNCaP cell membranes and in PC-3 cells transfected with PSM cDNA and also the characterization of PSM mRNA expression in human tissues, and in response to steroid hormones.

5

Brief Description of Figures

Figure 1:   Signal in lane 2 represent the 100kD PSM antigen. The EGFr was used as the positive control and is shown in lane 1. Incubation with rabbit antimouse (RAM) antibody alone served as negative control and is shown in lane 3.

Figure 2 A-D: Upper two photos show LNCaP cytospins staining positively for PSM antigen. Lower left in DU-145 and lower right is PC-3 cytospin, both negative for PSM antigen expression.

Figure 3 A-D: Upper two panels are human prostate sections (BPH) staining positively for PSM antigen. The lower two panels show invasive prostate carcinoma human sections staining positively for expression of the PSM antigen.

Figure 4:   100kD PSM antigen following immunoprecipitation of ^{35}S-Methionine labelled LNCaP cells with Cyt-356 antibody.

Figure 5:   3% agarose gels stained with Ethidium bromide revealing PCR products obtained using the degenerate PSM antigen primers. The arrow points to sample IN-20, which is a 1.1 kb PCR product which we later confirmed to be a partial cDNA coding for

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the PSM gene.

Figur 6 A-B: 2% agarose gels of plasmid DNA resulting from TA cloning of PCR products. Inserts are excised from the PCR II vector (Invitrogen Corp.) by digestion with EcoRI. 1.1 kb PSM gene partial cDNA product is shown in lane 3 of gel 1.

Figure 7: Autoradiogram showing size of cDNA represented in applicants' LNCaP library using M-MLV reverse transcriptase.

Figure 8: Restriction analysis of full-length clones of PSM gene obtained after screening cDNA library. Samples have been cut with Not I and Sal I restriction enzymes to liberate the insert.

Figure 9: Plasmid Southern autoradiogram of full length PSM gene clones. Size is approximately 2.7 kb.

Figure 10: Northern blot revealing PSM expression limited to LNCaP prostate cancer line and H26 Ras-transfected LNCaP cell line. PC-3, DU-145, T-24, SKRC-27, HELA, MCF-7, HL-60, and others were are all <u>negative</u>.

Figure 11: Autoradiogram of Northern analysis revealing expression of 2.8 kb PSM message unique to the LNCaP cell line (lane 1), and absent from the DU-145 (lane 2) and PC-3 cell lines (lane 3). RNA size ladder is shown on the left (kb), and 28S and 18S ribosomal RNA bands are indicated on the

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right.

Figure 12 A-B: Results of PCR of human prostate tissues
using PSM gene primers.   Lanes are
numbered from left to right. Lane 1,
LNCaP; Lane 2, H26; Lane 3, DU-145; Lane
4, Normal Prostate; Lane 5, BPH; Lane 6,
Prostate Cancer; Lane 7, BPH; Lane 8,
Normal; Lane 9, BPH; Lane 10, BPH; Lane
11, BPH; Lane 12, Normal; Lane 13, Normal;
Lane 14, Cancer; Lane 15, Cancer; Lane 16,
Cancer; Lane 17, Normal; Lane 18, Cancer;
Lane 19, IN-20 Control; Lane 20, PSM cDNA

Figure 13:   Isoelectric point of PSM antigen (non-
glycosylated)

Figure 14:1-8   Secondary structure of PSM antigen

Figure 15:A-B   A. Hydrophilicity plot of PSM antigen
B. Prediction   of   membrane   spanning
segments

Figure 16:1-11   Homology with chicken, rat and human
transferrin receptor sequence.

Figure 17A-C: Immunohistochemical   detection   of   PSM
antigen expression in prostate cell lines.
Top panel reveals uniformly high level of
expression in LNCaP cells; middle panel
and lower panel are DU-145 and PC-3 cells
respectively, both negative.

Figure 18:   Autoradiogram of protein gel revealing
products   of   PSM   coupled   *in-vitro*

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transcription/translation.   Non-
glycosylated PSM polypeptide is seen at 84
kDa   (lane   1)   and   PSM   glycoprotein
synthesized   following   the   addition   of
5   microsomes is seen at 100 kDa (lane 2).

Figure 19:   Western   Blot   analysis   detecting   PSM
expression   in   transfected   non-PSM
expressing   PC-3   cells.   100   kDa   PSM
10   glycoprotein   species   is   clearly   seen   in
LNCaP   membranes   (lane   1),   LNCaP   crude
lysate   (lane   2),   and   PSM-transfected   PC-3
cells   (lane   4),   but   is   undetectable   in
native PC-3 cells (lane 3).

15
Figure 20:   Autoradiogram of ribonuclease protection
gel   assaying   for   PSM   mRNA   expression   in
normal   human   tissues.   Radiolabeled   1   kb
DNA   ladder   (Gibco-BRL)   is   shown   in   lane   1.
20   Undigested   probe   is   400   nucleotides   (lane
2),   expected   protected   PSM   band   is   350
nucleotides,   and   tRNA   control   is   shown
(lane   3).   A   strong   signal   is   seen   in
human   prostate   (lane   11),   with   very   faint,
25   but   detectable   signals   seen   in   human   brain
(lane   4)   and   human   salivary   gland   (lane
12).

Figure 21:   Autoradiogram of ribonuclease protection
30   gel   assaying   for   PSM   mRNA   expression   in
LNCaP   tumors   grown   in   nude   mice,   and   in
human prostatic tissues. ^{32}P-labeled   1   kb
DNA   ladder   is   shown   in   lane   1.   298
nucleotide undigested probe is shown (lane

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2), and tRNA control is shown (lane 3).
PSM mRNA expression is clearly detectable
in LNCaP cells (lane 4), orthotopically
grown LNCaP tumors in nude mice with and
without matrigel (lanes 5 and 6), and
subcutaneously implanted and grown LNCaP
tumors in nude mice (lane 7). PSM mRNA
expression is also seen in normal human
prostate (lane 8), and in a moderately
differentiated human prostatic
adenocarcinoma (lane 10). Very faint
expression is seen in a sample of human
prostate tissue with benign hyperplasia
(lane 9).

Figure 22: Ribonuclease protection assay for PSM
expression in LNCaP cells treated with
physiologic doses of various steroids for
24 hours. ^{32}P-labeled DNA ladder is shown
in lane 1. 298 nucleotide undigested
probe is shown (lane 2), and tRNA control
is shown (lane 3). PSM mRNA expression is
highest in untreated LNCaP cells in
charcoal-stripped media (lane 4).
Applicant see significantly diminished PSM
expression in LNCaP cells treated with
DHT (lane 5), Testosterone (lane 6),
Estradiol (lane 7), and Progesterone (lane
8), with little response to Dexamethasone
(lane 9).

Figure 23: Data illustrating results of PSM DNA and
RNA presence in transfect Dunning cell
lines employing Southern and Northern

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blotting techniques

Figure 24:A-B   Figure A indicates the power of cytokine
                transfected cells to teach unmodified
5   cells. Administration was directed to the
                parental flank or prostate cells. The
                results indicate the microenvironment
                considerations.

10   Figure B indicates actual potency at a
                particular site. The tumor was implanted
                in prostate cells and treated with immune
                cells at two different sites.

15   Figure 25:A-B   Relates potency of cytokines in inhibiting
                growth of primary tumors. Animals
                administered un-modified parental tumor
                cells and administered as a vaccine
                transfected cells. Following
20   prostatectomy of rodent tumor results in
                survival increase.

     Figure 26:   PCR amplification with nested primers
                improved our level of detection of
25   prostatic cells from approximately one
                prostatic cell per 10,000 MCF-7 cells to
                better than one cell per million MCF-7
                cells, using either PSA.

30   Figure 27:   PCR amplification with nested primers
                improved our level of detection of
                prostatic cells from approximately one
                prostatic cell per 10,000 MCF-7 cells to
                better than one cell per million MCF-7
35   cells, using PSM-derived primers.

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Figure 28:   A representative ethidium stained gel
              photograph for PSM-PCR. Samples run in
              lane A represent PCR products generated
              from the outer primers and samples in
5   lanes labeled B are products of inner
              primer pairs.

Figure 29:   PSM Southern blot autoradiograph. The
              sensitivity of the Southern blot analysis
10   exceeded that of ethidium staining, as can
              be seen in several samples where the outer
              product is not visible on figure 3 A-D,
              but is detectable by Southern blotting as
              shown in figure 4.

15
Figure 30:   Characteristics of the 16 patients
              analyzed with respect to their clinical
              stage, treatment, serum PSA and PAP
              values, and results of assay.

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Summary of the Invention

This invention provides an isolated mammalian nucleic acid molecule encoding a mammalian prostate-specific membrane (PSM) antigen. The isolated mammalian nucleic acid may be DNA, cDNA or RNA.

This invention also provides nucleic acid molecule comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding the PSM antigen. The nucleic acid molecule may either be DNA or RNA.

This invention provides nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule which is complementary to the nucleic acid molecule encoding a mammalian prostate-specific membrane antigen.

This invention further provides a method of detecting expression of the PSM antigen which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a labelled PSM antigen specific nucleic acid molecule under hybridizing conditions, determining the presence of mRNA hybridized to the probe, and thereby detecting the expression of the PSM antigen by the cell. The PSM antigen in tissue sections may be similarly detected.

This invention provides isolated nucleic acid molecule of PSM antigen operatively linked to a promoter of RNA transcription. This invention further provides a vector which comprises an isolated mammalian nucleic acid molecule of PSM antigen.

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This invention further provides a host vector system for
the production of a polypeptide having the biological
activity of a mammalian PSM antigen which comprises the
vector comprising the mammalian nucleic acid molecule
5   encoding a mammalian PSM antigen and a suitable host.
The suitable host for the expression of PSM antigen may
be a bacterial cell, insect cell, or mammalian cell.

This invention also provides a method of producing a
10   polypeptide having the biological activity of a mammalian
PSM antigen which comprises growing the host cell of
vector system having a vector comprising the isolated
mammalian nucleic acid molecule encoding a mammalian PSM
antigen and a suitable host under suitable conditions
15   permitting production of the polypeptide and recovery of
the polypeptide so produced.

This invention provides a method for determining whether
a ligand can bind to a mammalian PSM antigen which
20   comprises contacting a mammalian cell having an isolated
mammalian DNA molecule encoding a mammalian PSM antigen
with the ligand under conditions permitting binding of
ligands to the mammalian PSM antigen, and determining
whether the ligand binds to a mammalian PSM antigen.
25   This invention further provides ligands which bind to PSM
antigen.

This invention provides purified mammalian PSM antigen.
This invention also provides a polypeptide encoded by the
30   isolated mammalian nucleic acid molecule encoding a
mammalian PSM antigen. This invention further provides
a method to identify and purify ligands of mammalian PSM
antigen.

35   This invention further provides a method to produce both

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polyclonal and monoclonal antibody using purified PSM antigens or polypeptides encoded by an isolated mammalian nucleic acid molecule encoding a mammalian PSM antigen.

5 This invention provides polyclonal and monoclonal antibody most likely but not limited to directed either to peptide Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID No. 35), or Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No. 36) or Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No. 37) of the PSM antigen.

10

This invention provides a therapeutic agent comprising an antibody directed against a mammalian PSM antigen and a cytotoxic agent conjugated thereto.

15 This invention also provides a method of imaging prostate cancer in human patients which comprises administering to the patient at least one antibody directed against PSM antigen, capable of binding to the cell surface of the prostate cancer cell and labeled with an imaging agent
20 under conditions so as to form a complex between the monoclonal antibody and the cell surface PSM antigen. This invention further provides a composition comprising an effective imaging amount of the antibody directed against PSM antigen and a pharmaceutically acceptable
25 carrier.

This invention further provides a method of imaging prostate cancer in human patients which comprises administering to the patient multiple antibodies directed
30 towards different PSM epitopes.

The invention also provides a method of imaging prostate cancer in human patients which comprises administering to the patient at least one ligand, capable of binding to
35 the cell surface of the prostate cancer cell and labelled

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with an imaging agent under conditions so as to form a complex between the ligand and the cell surface PSM antigen. This invention further provides a composition comprising an effective imaging amount of PSM antigen and
5   a pharmaceutically acceptable carrier.

This invention provides an immunoassay for measuring the amount of the PSM antigen in a biological sample, e.g. serum, comprising steps of a) contacting the biological
10   sample with at least one PSM antibody to form a complex with said antibody and the PSM antigen, and b) measuring the amount of PSM antigen in said biological sample by measuring the amount of said complex.

15   This invention also provides an immunoassay for measuring the amount of the PSM antigen in a biological sample comprising steps of a) contacting the biological sample with at least one PSM ligand to form a complex with said ligand and the PSM antigen, and b) measuring the amount
20   of the PSM antigen in said biological sample by measuring the amount of said complex.

This invention provides a method to purify mammalian PSM antigen comprising steps of: a) coupling the antibody
25   directed against PSM antigen to a solid matrix; b) incubating the coupled antibody of a) with a cell lysate containing PSM antigen under the condition permitting binding of the antibody and PSM antigen; c) washing the coupled solid matrix to eliminate impurities and d)
30   eluting the PSM antigen from the bound antibody.

This invention further provides transgenic nonhuman mammals which comprises an isolated nucleic acid molecule of PSM antigen. This invention also provides a
35   transgenic nonhuman mammal whose genome comprises

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antisense DNA complementary to DNA encoding a mammalian PSM antigen so placed as to be transcribed into antisense mRNA complementary to mRNA encoding the PSM antigen and which hybridizes to mRNA encoding the PSM antigen thereby reducing its translation.

This invention provides a method of suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells comprising introducing a DNA molecule encoding a prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell of a subject, in a way that expression of the prostate specific membrane antigen is under the control of the regulatory element, thereby suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells.

This invention provides a method of suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells, comprising introducing a DNA molecule encoding a prostate specific membrane antigen operatively linked to a 5' regulatory element coupled with a therapeutic DNA into a tumor cell of a subject, thereby suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells.

This invention provides a therapeutic vaccine for preventing human prostate tumor growth or stimulation of prostate tumor cells in a subject, comprising administering an effective amount to the prostate cell, and a pharmaceutical acceptable carrier, thereby preventing the tumor growth or stimulation of tumor cells

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in the subject.

This invention provides a method of detecting hematogenous micrometastic tumor cells of a subject,
5   comprising (A) performing nested polymerase chain reaction (PCR) on blood, bone marrow, or lymph node samples of the subject using the prostate specific membrane antigen primers, and (B) verifying micrometastases by DNA sequencing and Southern analysis,
10   thereby detecting hematogenous micrometastic tumor cells of the subject.

This invention provides a method of abrogating the mitogenic response due to transferrin, comprising
15   introducing a DNA molecule encoding prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell, the expression of which gene is directly associated with a defined pathological effect within a multicellular organism, thereby abrogating
20   mitogen response due to transferrin.

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D tailed Description of the Invention

Throughout this application, references to specific
nucleotides are to nucleotides present on the coding
5   strand of the nucleic acid.   The following standard
abbreviations are used throughout the specification to
indicate specific nucleotides:

C=cytosine   A=adenosine
T=thymidine   G=guanosine
10

A "gene" means a nucleic acid molecule, the sequence of
which includes all the information required for the
normal regulated production of a particular protein,
including the structural coding sequence, promoters and
15   enhancers.

This invention provides an isolated mammalian nucleic
acid encoding a mammalian prostate-specific membrane
(PSM) antigen.
20

This invention further provides an isolated mammalian DNA
molecule of an isolated mammalian nucleic acid molecule
encoding a mammalian prostate-specific membrane antigen.
This invention also provides an isolated mammalian cDNA
25   molecule encoding a mammalian prostate-specific membrane
antigen.   This invention provides an isolated mammalian
RNA molecule encoding a mammalian prostate-specific
membrane antigen.

30   In the preferred embodiment of this invention, the
isolated nucleic sequence is cDNA from human as shown in
sequence ID number 1.   This human sequence was submitted
to GenBank (Los Alamos National Laboratory, Los Alamos,
New Mexico) with Accession Number, M99487 and the
35   description as PSM, Homo sapiens, 2653 base-pairs.

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This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of PSM antigen, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs and cDNAs which hybridize to the DNA and cDNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The DNA molecules of the subject invention also include DNA molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

The DNA molecules described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured

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growth of such host cells capable of expression of the polypeptide and related products.

Moreover, the isolated mammalian nucleic acid molecules
5   encoding a mammalian prostate-specific membrane antigen are useful for the development of probes to study the tumorigenesis of prostate cancer.

This invention also provides nucleic acid molecules of at
10 least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule encoding the prostate-specific membrane antigen.

This nucleic acid molecule produced can either be DNA or
15 RNA.   As   used   herein,   the   phrase   "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.
20
This nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule encoding the prostate-specific membrane antigen can be used as a probe.   Nucleic acid
25 probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe.   DNA probe molecules
30 may be produced by insertion of a DNA molecule which encodes PSM antigen into suitable vectors,   such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells,   replication in the transformed bacterial host cells and harvesting of the
35 DNA probes,   using methods well known in the art.

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Alternatively, probes may be generated chemically from DNA synthesizers.

RNA probes may be generated by inserting the PSM antigen molecule downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized PSM antigen fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

This invention also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule which is complementary to the mammalian nucleic acid molecule encoding a mammalian prostate-specific membrane antigen. This molecule may either be a DNA or RNA molecule.

The current invention further provides a method of detecting the expression of a mammalian PSM antigen expression in a cell which comprises obtaining total mRNA from the cell, contacting the mRNA so obtained with a labelled nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence of the nucleic acid molecule encoding a mammalian PSM antigen under hybridizing conditions, determining the presence of mRNA hybridized to the molecule and thereby detecting the expression of the mammalian prostate-specific membrane antigen in the cell. The nucleic acid molecules synthesized above may be used to detect expression of a PSM antigen by detecting the presence of mRNA coding for the PSM antigen. Total mRNA from the cell may be isolated by many procedures well known to a person of ordinary skill in the art. The hybridizing

conditions of the labelled nucleic acid molecules may be
determined by routine experimentation well known in the
art. The presence of mRNA hybridized to the probe may be
determined by gel electrophoresis or other methods known

5   in the art. By measuring the amount of the hybrid made,
the expression of the PSM antigen by the cell can be
determined. The labelling may be radioactive. For an
example, one or more radioactive nucleotides can be
incorporated in the nucleic acid when it is made.

10

In one embodiment of this invention, nucleic acids are
extracted by precipitation from lysed cells and the mRNA
is isolated from the extract using an oligo-dT column
which binds the poly-A tails of the mRNA molecules (13).

15   The mRNA is then exposed to radioactively labelled probe
on a nitrocellulose membrane, and the probe hybridizes to
and thereby labels complementary mRNA sequences. Binding
may be detected by luminescence autoradiography or
scintillation counting. However, other methods for

20   performing these steps are well known to those skilled in
the art, and the discussion above is merely an example.

This invention further provides another method to detect
expression of a PSM antigen in tissue sections which

25   comprises contacting the tissue sections with a labelled
nucleic acid molecule of at least 15 nucleotides capable
of specifically hybridizing with a sequence of nucleic
acid molecules encoding a mammalian PSM antigen under
hybridizing conditions, determining the presence of mRNA

30   hybridized to the molecule and thereby detecting the
expression of the mammalian PSM antigen in tissue
sections. The probes are also useful for in-situ
hybridization or in order to locate tissues which express
this gene, or for other hybridization assays for the

35   presence of this gene or its mRNA in various biological

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tissues.   The in-situ hybridization using a labelled
nucleic acid molecule is well known in the art.
Essentially, tissue sections are incubated with the
labelled nucleic acid molecule to allow the hybridization
to occur.   The molecule will carry a marker for the
detection because it is "labelled", the amount of the
hybrid will be determined based on the detection of the
amount of the marker and so will the expression of PSM
antigen.

This invention further provides isolated PSM antigen
nucleic acid molecule operatively linked to a promoter of
RNA transcription. The isolated PSM antigen sequence can
be linked to vector systems. Various vectors including
plasmid vectors, cosmid vectors, bacteriophage vectors
and other viruses are well known to ordinary skilled
practitioners. This invention further provides a vector
which comprises the isolated nucleic acid molecule
encoding for the PSM antigen.

As an example to obtain these vectors, insert and vector
DNA can both be exposed to a restriction enzyme to create
complementary ends on both molecules which base pair with
each other and are then ligated together with DNA ligase.
Alternatively, linkers can be ligated to the insert DNA
which correspond to a restriction site in the vector DNA,
which is then digested with the restriction enzyme which
cuts at that site. Other means are also available and
known to an ordinary skilled practitioner.

In an embodiment, the PSM sequence is cloned in the Not
I/Sal I site of pSPORT/vector (Gibco® - BRL). This
plasmid, p55A-PSM, was deposited on August 14, 1992 with
the American Type Culture Collection (ATCC), 12301
Parklawn Drive, Rockville, Maryland 20852, U.S.A. under

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the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. Plasmid, p55A-PSM, was accorded ATCC Accession Number 75294.

5

This invention further provides a host vector system for the production of a polypeptide having the biological activity of the prostate-specific membrane antigen. These vectors may be transformed into a suitable host
10   cell to form a host cell vector system for the production of a polypeptide having the biological activity of PSM antigen.

Regulatory elements required for expression include
15   promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start
20   codon AUG (14). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained
25   commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the PSM antigen.

30

This invention further provides an isolated DNA or cDNA molecule described hereinabove wherein the host cell is selected from the group consisting of bacterial cells (such as E.coli), yeast cells, fungal cells, insect cells
35   and animal cells. Suitable animal cells include, but are

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not limited to Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

This invention further provides a method of producing a polypeptide having the biological activity of the prostate-specific membrane antigen which comprising growing host cells of a vector system containing the PSM antigen sequence under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

This invention provides a mammalian cell comprising a DNA molecule encoding a mammalian PSM antigen, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a mammalian PSM antigen and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding the mammalian PSM antigen as to permit expression thereof.

Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk⁻ cells, Cos cells, etc. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, electroporation or DNA encoding the mammalian PSM antigen may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a mammalian PSM antigen.

This invention provides a method for determining whether

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a ligand can bind to a mammalian prostate-specific membrane antigen which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian prostate-specific membrane antigen with the
5   ligand under conditions permitting binding of ligands to the mammalian prostate-specific membrane antigen, and thereby determining whether the ligand binds to a mammalian prostate-specific membrane antigen.

10   This invention further provides ligands bound to the mammalian PSM antigen.

     This invention also provides a therapeutic agent comprising a ligand identified by the above-described
15   method and a cytotoxic agent conjugated thereto. The cytotoxic agent may either be a radioisotope or a toxin. Examples of radioisotopes or toxins are well known to one of ordinary skill in the art.

20   This invention also provides a method of imaging prostate cancer in human patients which comprises administering to the patients at least one ligand identified by the above-described method, capable of binding to the cell surface of the prostate cancer cell and labelled with an imaging
25   agent under conditions permitting formation of a complex between the ligand and the cell surface PSM antigen. This invention further provides a composition comprising an effective imaging agent of the PSM antigen ligand and a pharmaceutically acceptable carrier. Pharmaceutically
30   acceptable carriers are well known to one of ordinary skill in the art. For an example, such a pharmaceutically acceptable carrier can be physiological saline.

35   Also provided by this invention is a purified mammalian

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PSM antigen. As used herein, the term "purified prostate-specific membrane antigen" shall mean isolated naturally-occurring prostate-specific membrane antigen or protein (purified from nature or manufactured such that the primary, secondary and tertiary conformation, and posttranslational modifications are identical to naturally-occurring material) as well as non-naturally occurring polypeptides having a primary structural conformation (i.e. continuous sequence of amino acid residues). Such polypeptides include derivatives and analogs.

This invention further provides a polypeptide encoded by the isolated mammalian nucleic acid sequence of PSM antigen.

It is believed that there may be natural ligand interacting with the PSM antigen. This invention provides a method to identify such natural ligand or other ligand which can bind to the PSM antigen. A method to identify the ligand comprises a) coupling the purified mammalian PSM antigen to a solid matrix, b) incubating the coupled purified mammalian PSM protein with the potential ligands under the conditions permitting binding of ligands and the purified PSM antigen; c) washing the ligand and coupled purified mammalian PSM antigen complex formed in b) to eliminate the nonspecific binding and impurities and finally d) eluting the ligand from the bound purified mammalian PSM antigen. The techniques of coupling proteins to a solid matrix are well known in the art. Potential ligands may either be deduced from the structure of mammalian PSM or by other empirical experiments known by ordinary skilled practitioners. The conditions for binding may also easily be determined and protocols for carrying such experimentation have long

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been well documented (15). The ligand-PSM antigen complex will be washed. Finally, the bound ligand will be eluted and characterized. Standard ligands characterization techniques are well known in the art.

The above method may also be used to purify ligands from any biological source. For purification of natural ligands in the cell, cell lysates, serum or other biological samples will be used to incubate with the mammalian PSM antigen bound on a matrix. Specific natural ligand will then be identified and purified as above described.

With the protein sequence information, antigenic areas may be identified and antibodies directed against these areas may be generated and targeted to the prostate cancer for imaging the cancer or therapies.

This invention provides an antibody directed against the amino acid sequence of a mammalian PSM antigen.

This invention provides a method to select specific regions on the PSM antigen to generate antibodies. The protein sequence may be determined from the PSM DNA sequence. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer of the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Usually, the hydrophilic regions will be more immunogenic than the hydrophobic regions. Therefore the hydrophilic amino acid sequences may be

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selected and used to generate antibodies specific to
mammalian PSM antigen.   For an example, hydrophilic
sequences   of   the   human   PSM   antigen   shown   in
hydrophilicity plot of Figure 16 may be easily selected.
5   The selected peptides may be prepared using commercially
available machines.   As an alternative, DNA, such as a
cDNA or a fragment thereof, may be cloned and expressed
and the resulting polypeptide recovered and used as an
immunogen.
10

Polyclonal   antibodies   against   these   peptides   may   be
produced   by   immunizing   animals   using   the   selected
peptides.   Monoclonal   antibodies   are   prepared   using
hybridoma technology by fusing antibody producing B cells
15   from immunized animals with myeloma cells and selecting
the resulting hybridoma cell line producing the desired
antibody.   Alternatively, monoclonal antibodies may be
produced by _in vitro_ techniques known to a person of
ordinary skill in the art.   These antibodies are useful
20   to detect the expression of mammalian PSM antigen in
living animals, in humans, or in biological tissues or
fluids isolated from animals or humans.

In one embodiment, peptides Asp-Glu-Leu-Lys-Ala-Glu (SEQ
25   ID No. 35), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No. 36) and
Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No. 37) of human PSM
antigen are selected.

This invention further provides polyclonal and monoclonal
30   antibody(ies) against peptides Asp-Glu-Leu-Lys-Ala-Glu
(SEQ ID No. 35), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No. 36)
and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No. 37).

This invention provides a therapeutic agent comprising
35   antibodies or ligand(s) directed against PSM antigen and

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a cytotoxic agent conjugated thereto or antibodies linked enzymes which activate prodrug to kill the tumor. The cytotoxic agent may either be a radioisotope or toxin.

5   This invention provides a method of imaging prostate cancer in human patients which comprises administering to the patient the monoclonal antibody directed against the peptide of the mammalian PSM antigen capable of binding to the cell surface of the prostate cancer cell and
10   labeled with an imaging agent under conditions permitting formation of a complex between the monoclonal antibody and the cell surface prostate-specific membrane antigen. The imaging agent is a radioisotope such as Indium[111].

15   This invention further provides a prostate cancer specific imaging agent comprising the antibody directed against PSM antigen and a radioisotope conjugated thereto.

20   This invention also provides a composition comprising an effective imaging amount of the antibody directed against the PSM antigen and a pharmaceutically acceptable carrier. The methods to determine effective imaging amounts are well known to a skilled practitioner. One
25   method is by titration using different amounts of the antibody.

      This invention further provides an immunoassay for measuring the amount of the prostate-specific membrane
30   antigen in a biological sample comprising steps of a) contacting the biological sample with at least one antibody directed against the PSM antigen to form a complex with said antibody and the prostate-specific membrane antigen, and b) measuring the amount of the

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prostate-specific membrane antigen in said biological
sample by measuring the amount of said complex.   One
example of the biological sample is a serum sample.

5   This invention provides a method to purify mammalian
prostate-specific membrane antigen comprising steps of a)
coupling the antibody directed against the PSM antigen to
a solid matrix; b) incubating the coupled antibody of a)
with lysate containing prostate-specific membrane antigen
10   under the condition which the antibody and prostate
membrane specific can bind; c) washing the solid matrix
to eliminate impurities and d) eluting the prostate-
specific membrane antigen from the coupled antibody.

15   This invention also provides a transgenic nonhuman mammal
which comprises the isolated nucleic acid molecule
encoding a mammalian PSM antigen.   This invention further
provides a transgenic nonhuman mammal whose genome
comprises antisense DNA complementary to DNA encoding a
20   mammalian prostate-specific membrane antigen so placed as
to be transcribed into antisense mRNA complementary to
mRNA encoding the prostate-specific membrane antigen and
which hybridizes to mRNA encoding the prostate specific
antigen thereby reducing its translation.
25
Animal model systems which elucidate the physiological
and behavioral roles of mammalian PSM antigen are
produced by creating transgenic animals in which the
expression of the PSM antigen is either increased or
30   decreased, or the amino acid sequence of the expressed
PSM antigen is altered, by a variety of techniques.
Examples of these techniques include, but are not limited
to: 1) Insertion of normal or mutant versions of DNA
encoding a mammalian PSM antigen, by microinjection,
35   electroporation, retroviral transfection or other means

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well known to those skilled in the art, into appropriate
fertilized embryos in order to produce a transgenic
animal (16) or 2) Homologous recombination (17) of mutant
or normal, human or animal versions of these genes with
5   the native gene locus in transgenic animals to alter the
regulation of expression or the structure of these PSM
antigen sequences.   The technique of homologous
recombination is well known in the art. It replaces the
native gene with the inserted gene and so is useful for
10   producing an animal that cannot express native PSM
antigen but does express, for example, an inserted mutant
PSM antigen, which has replaced the native PSM antigen in
the animal's genome by recombination, resulting in
underexpression of the transporter. Microinjection adds
15   genes to the genome, but does not remove them, and so is
useful for producing an animal which expresses its own
and added PSM antigens, resulting in overexpression of
the PSM antigens.

20   One means available for producing a transgenic animal,
with a mouse as an example, is as follows: Female mice
are mated, and the resulting fertilized eggs are
dissected out of their oviducts. The eggs are stored in
an appropriate medium such as M2 medium (16). DNA or
25   cDNA encoding a mammalian PSM antigen is purified from a
vector by methods well known in the art. Inducible
promoters may be fused with the coding region of the DNA
to provide an experimental means to regulate expression
of the trans-gene. Alternatively or in addition, tissue
30   specific regulatory elements may be fused with the coding
region to permit tissue-specific expression of the trans-
gene. The DNA, in an appropriately buffered solution, is
put into a microinjection needle (which may be made from
capillary tubing using a pipet puller) and the egg to be
35   injected is put in a depression slide.   The needle is

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inserted into the pronucleus of the egg, and the DNA
solution is injected.   The injected egg is then
transferred into the oviduct of a pseudopregnant mouse (a
mouse stimulated by the appropriate hormones to maintain
5   pregnancy but which is not actually pregnant), where it
proceeds to the uterus, implants, and develops to term.
As noted above, microinjection is not the only method for
inserting DNA into the egg cell, and is used here only
for exemplary purposes.

10

Another use of the PSM antigen sequence is to isolate
homologous gene or genes in different mammals. The gene
or genes can be isolated by low stringency screening of
either cDNA or genomic libraries of different mammals
15   using probes from PSM sequence.   The positive clones
identified will be further analyzed by DNA sequencing
techniques which are well known to an ordinary person
skilled in the art.   For example, the detection of
members of the protein serine kinase family by homology
20   probing (18).

This invention provides a method of suppressing or
modulating metastatic ability of prostate tumor cells,
prostate tumor growth or elimination of prostate tumor
25   cells comprising introducing a DNA molecule encoding a
prostate specific membrane antigen operatively linked to
a 5' regulatory element into a tumor cell of a subject,
in a way that expression of the prostate specific
membrane antigen is under the control of the regulatory
30   element, thereby suppressing or modulating metastatic
ability of prostate tumor cells, prostate tumor growth or
elimination of prostate tumor cells.   The subject may be
a mammal or more specifically a human.

35   In one embodiment, the DNA molecule encoding prostate

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specific membrane antigen operatively linked to a 5'
regulatory element forms part of a transfer vector which
is inserted into a cell or organism. In addition the
vector is capable or replication and expression of
5   prostate specific membrane antigen. The DNA molecule
encoding prostate specific membrane antigen can be
integrated into a genome of a eukaryotic or prokaryotic
cell or in a host cell containing and/or expressing a
prostate specific membrane antigen.

10

Further, the DNA molecule encoding prostate specific
membrane antigen may be introduced by a bacterial, viral,
fungal, animal, or liposomal delivery vehicle. Other
means are also available and known to an ordinary skilled
15 practitioner.

Further, the DNA molecule encoding a prostate specific
membrane antigen operatively linked to a promoter or
enhancer. A number of viral vectors have been described
20 including those made from various promoters and other
regulatory elements derived from virus sources.
Promoters consist of short arrays of nucleic acid
sequences that interact specifically with cellular
proteins involved in transcription. The combination of
25 different recognition sequences and the cellular
concentration of the cognate transcription factors
determines the efficiency with which a gene is
transcribed in a particular cell type.

30 Examples of suitable promoters include a viral promoter.
Viral promoters include: adenovirus promoter, an simian
virus 40 (SV40) promoter, a cytomegalovirus (CMV)
promoter, a mouse mammary tumor virus (MMTV) promoter, a
Malony murine leukemia virus promoter, a murine sarcoma
35 virus promoter, and a Rous sarcoma virus promoter.

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Further, another suitable promoter is a heat shock promoter. Additionally, a suitable promoter is a bacteriophage promoter. Examples of suitable bacteriophage promoters include but not limited to, a T7 promoter, a T3 promoter, an SP6 promoter, a lambda promoter, a baculovirus promoter.

Also suitable as a promoter is an animal cell promoter such as an interferon promoter, a metallothionein promoter, an immunoglobulin promoter. A fungal promoter is also a suitable promoter. Examples of fungal promoters include but are not limited to, an ADC1 promoter, an ARG promoter, an ADH promoter, a CYC1 promoter, a CUP promoter, an ENO1 promoter, a GAL promoter, a PHO promoter, a PGK promoter, a GAPDH promoter, a mating type factor promoter. Further, plant cell promoters and insect cell promoters are also suitable for the methods described herein.

This invention provides a method of suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells, comprising introducing a DNA molecule encoding a prostate specific membrane antigen operatively linked to a 5' regulatory element coupled with a therapeutic DNA into a tumor cell of a subject, thereby suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells. The subject may be a mammal or more specifically a human.

Further, the therapeutic DNA which is coupled to the DNA molecule encoding a prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell may code for a cytokine, viral antigen, or a pro-drug activating enzyme. Other means are also available and known

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to an ordinary skilled practitioner.

5   The cytokine used may be interl ukin-2, interleukin-12, interferon alpha, beta or gamma, granulocytic macrophage - colony stimulating factor, or other immunity factors.

In addition, this invention provides a prostate tumor cell, comprising a DNA molecule isolated from mammalian nucleic
10   acid encoding a mammalian prostate-specific membrane antigen under the control of a prostate specific membrane antigen operatively linked to a 5' regulatory element.

As used herein, DNA molecules include complementary DNA
15   (cDNA), synthetic DNA, and genomic DNA.

This invention provides a therapeutic vaccine for preventing human prostate tumor growth or stimulation of prostate tumor cells in a subject, comprising administering an effective
20   amount to the prostate cell, and a pharmaceutical acceptable carrier, thereby preventing the tumor growth or stimulation of tumor cells in the subject. Other means are also available and known to an ordinary skilled practitioner.
25
This invention provides a method of detecting hematogenous micrometastic tumor cells of a subject, comprising (A) performing nested polymerase chain reaction (PCR) on blood, bone marrow or lymph node samples of the subject using the
30   prostate specific membrane antigen primers, and (B) verifying micrometastases by DNA sequencing and Southern analysis, thereby detecting hematogenous micrometastic tumor cells of the subject. The subject may be a mammal or more specifically a human.
35
The micrometastatic tumor cell may be a prostatic cancer

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and the DNA primers may be deriv d from prostate specific antigen. Further, the subject may be administered with simultaneously an effective amount of hormones, so as to increase expression of prostate specific membrane antigen.

5

This invention provides a method of abrogating the mitogenic response due to transferrin, comprising introducing a DNA molecule encoding prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor
10 cell, the expression of which gene is directly associated with a defined pathological effect within a multicellular organism, thereby abrogating mitogen response due to transferrin. The tumor cell may be a prostate cell.

15 This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow
20 thereafter.

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Experimental Details

First Series of Experiments

5   Materials and Methods
        The approach for cloning the gene involved purification of
        the antigen in large quantities by immunoprecipitation, and
        microsequencing of several internal peptides for use in
        synthesizing   degenerate   oligonucleotide   primers   for
10   subsequent use in the polymerase chain reaction (19, 20).
        A partial cDNA was amplified as a PCR product and this was
        used as a homologous probe to clone the full-length cDNA
        molecule from a LNCaP (Lymph Node Carcinoma of Prostate)
        cell line cDNA plasmid library (8).   Early experiments
15   revealed to us that the CYT-356 antibody (9) was not capable
        of detecting the antigen produced in bacteria since the
        epitope was the glycosylated portion of the PSM antigen, and
        this   necessitated   our   more   difficult,   yet   elaborate
        approach.

20

        Western Analysis of the PSM Antigen
        Membrane proteins were isolated from cells by hypotonic
        lysis followed by centrifugation over a sucrose density
        gradient (21).   10-20μg of LNCaP, DU-145, and PC-3 membrane
25   proteins   were   electrophoresed   through   a   10%   SDS-PAGE
        resolving gel with a 4% stacking gel at 9-10 milliamps for
        16-18 hours.   Proteins were electroblotted onto PVDF
        membranes (Millipore® Corp.) in transfer buffer (48mM Tris
        base, 39mM Glycine, 20% Methanol) at 25 volts overnight at
30   4°C.   Membranes were blocked in TSB (0.15M NaCl, 0.01M Tris
        base, 5% BSA) for 30 minutes at room temperature followed by
        incubation with 10-15μg/ml of CYT-356 monoclonal antibody
        (Cytogen Corp.) for 2 hours.   Membranes were then incubated
        with   10-15μg/ml   of   rabbit   anti-mouse   immunoglobulin
35   (Accurate   Scientific)   for   1   hour   at   room   temperature

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followed by incubation with ^{125}I-Protein A (Amersham®) at $1x10^6$ cpm/ml at room temperature. Membranes were then washed and autoradiographed for 12-24 hours at -70°C (Figure 1).

5

Immunohistochemical Analysis of PSM Antigen Expression

The avidin-biotin method of immunohistochemical detection was employed to analyze both human tissue sections and cell lines for PSM Antigen expression (22). Cryostat-cut

10 prostate tissue sections (4-6μm thick) were fixed in methanol/acetone for 10 minutes. Cell cytospins were made on glass slides using 50,000 cells/100μl/slide. Samples were treated with 1% hydrogen peroxide in PBS for 10-15 minutes in order to remove any endogenous peroxidase

15 activity. Tissue sections were washed several times in PBS, and then incubated with the appropriate suppressor serum for 20 minutes. The suppressor serum was drained off and the sections or cells were then incubated with the diluted CYT-356 monoclonal antibody for 1 hour. Samples were then

20 washed with PBS and sequentially incubated with secondary antibodies (horse or goat immunoglobulins, 1:200 dilution for 30 minutes), and with avidin-biotin complexes (1:25 dilution for 30 minutes). DAB was used as a chromogen, followed by hematoxylin counterstaining and mounting.

25 Frozen sections of prostate samples and duplicate cell cytospins were used as controls for each experiment. As a positive control, the anti-cytokeratin monoclonal antibody CAM 5.2 was used following the same procedure described above. Tissue sections are considered by us to express the

30 PSM antigen if at least 5% of the cells demonstrate immunoreactivity. Our scoring system is as follows: 1 = <5%; 2 = 5-19%; 3 = 20-75%; and 4 = >75% positive cells. Homogeneity versus heterogeneity was accounted for by evaluating positive and negative cells in 3-5 high power

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light microscopic fields (400x), recording the percentage of
positive cells among 100-500 cells. The intensity of
immunostaining is graded on a 1+ to 4+ scale, where 1+
represents mild, 2-3+ represents moderate, and 4+ represents
5 intense immunostaining as compared to positive controls.

Immunoprecipitation of the PSM Antigen

80%-confluent LNCaP cells in 100mm petri dishes were starved
in RPMI media without methionine for 2 hours, after which
10 ^{35}S-Methionine was added at 100μCi/ml and the cells were
grown for another 16-18 hours. Cells were then washed and
lysed by the addition of 1ml of lysis buffer (1% Triton X-
100, 50mM Hepes pH 7.5, 10% glycerol, 150mM MgCl$_2$, 1mM PMSF,
and 1mM EGTA) with incubation for 20 minutes at 4°C.
15 Lysates were pre-cleared by mixing with Pansorbin® cells
(Calbiochem®) for 90 minutes at 4°C. Cell lysates were then
mixed with Protein A Sepharose® CL-4B beads (Pharmacia®)
previously bound with CYT-356 antibody (Cytogen Corp.) and
RAM antibody (Accurate Scientific) for 3-4 hours at 4°C.
20 12μg of antibody was used per 3mg of beads per petri dish.
Beads were then washed with HNTG buffer (20mM Hepes pH 7.5,
150mM NaCl, 0.1% Triton X-100, 10% glycerol, and 2mM Sodium
Orthovanadate), resuspended in sample loading buffer
containing ß-mercaptoethanol, denatured at 95°C for 5-10
25 minutes and run on a 10% SDS-PAGE gel with a 4° stacking gel
at 10 milliamps overnight. Gels were stained with Coomassie
Blue, destained with acetic acid/methanol, and dried down in
a vacuum dryer at 60°C. Gels were then autoradiographed for
16-24 hours at -70°C (Figure 2 A-D).

30

Large-Scale Immunoprecipitation and Peptide Sequencing

The procedure described above for immunoprecipitation was
repeated with 8 confluent petri dishes containing
approximately 6x10^7 LNCaP cells. The immunoprecipitation

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product was pooled and loaded into two lanes of a 10% SDS-PAGE gel and electrophoresed at 9-10 milliamps for 16 hours. Proteins were electroblotted onto Nitrocellulose BA-85 membranes (Schleicher and Schuell®) for 2 hours at 75 volts
5   at 4°C in transfer buffer. Membranes were stained with Ponceau Red to visualize the proteins and the 100kD protein band was excised, solubilized, and digested proteolytically with trypsin. HPLC was then performed on the digested sample on an Applied Biosystems Model 171C and clear
10   dominant peptide peaks were selected and sequenced by modified Edman degradation on a modified post liquid Applied Biosystems Model 477A Protein/Peptide Microsequencer (23). Sequencing data on all of the peptides is included within this document. We attempted to sequence the amino-terminus
15   of the PSM antigen by a similar method which involved purifying the antigen by immunoprecipitation and transfer via electro-blotting to a PVDF membrane (Millipore®). Protein was analyzed on an Applied Biosystems Model 477A Protein/Peptide Sequencer and the amino terminus was found
20   to be blocked, and therefore no sequence data could be obtained by this technique.

     PSM Antigen Peptide Sequences:

25   2T17 #5   SLYES(W)TK (SEQ ID No. 3)
     2T22 #9   (S)YPDGXNLPGG(g)VQR (SEQ ID No. 4)
     2T26 #3   FYDPMFK (SEQ ID No. 5)
     2T27 #4   IYNVIGTL(K) (SEQ ID No. 6)
     2T34 #6   FLYXXTQIPHLAGTEQNFQLAK (SEQ ID No. 7)
30   2T35 #2   G/PVILYSDPADYFAPD/GVK (SEQ ID No. 8, 9)
     2T38 #1   AFIDPLGLPDRPFYR (SEQ ID No. 10)
     2T46 #8   YAGESFPGIYDALFDIESK (SEQ ID No. 11)
     2T47 #7   TILFAS(W)DAEEFGXX(q)STE(e)A(E)..(SEQ ID No. 12)

35   Notes: X means that no residue could be identified at this

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position. Capital denotes identification but with a lower degree of confidence. (lower case) means residue present but at very low levels. ... indicates sequence continues but has dropped below detection limit.

5

All of these peptide sequences were verified to be unique after a complete homology search of the translated Genbank computer database.

10 <u>Degenerate PCR</u>

Sense and anti-sense 5'-unphosphorylated degenerate oligonucleotide primers 17 to 20 nucleotides in length corresponding to portions of the above peptides were synthesized on an Applied Biosystems Model 394A DNA

15 Synthesizer. These primers have degeneracies from 32 to 144. The primers used are shown below. The underlined amino acids in the peptides represent the residues used in primer design.

20 <u>Peptide 3</u>:   **FYDPMFK** (SEQ ID No. 5)

PSM Primer "A" TT(C or T) - TA(C or T) - GA(C or T) - CCX - ATG - TT (SEQ ID No. 13)

25 PSM Primer "B" AAC - ATX - GG(A or G) - TC(A or G) - TA(A or G) - AA (SEQ ID No. 14)

Primer A is sense primer and B is anti-sense. Degeneracy is 32-fold.

30

<u>Peptide 4</u>:   **IYNVIGTL(K)** (SEQ ID No. 6)

PSM Primer "C" AT(T or C or A) - TA(T or C) - AA(T or C) - GTX - AT(T or C or A) - GG (SEQ ID No. 15)

35

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PSM Primer "D" CC(A or T or G) - ATX - AC(G or A) - TT(A or G) - TA(A or G or T) - AT (SEQ ID No. 16)

Primer C is sense primer and D is anti-sense. Degeneracy is
5   144-fold.

Peptide 2:   G/PVILYSDPADYFAPD/GVK (SEQ ID No. 8,9)

PSM Primer "E" CCX - GCX - GA(T or C) - TA(T or C) - TT(T
10   or C) - GC (SEQ ID No. 17)

PSM Primer "F" GC(G or A) - AA(A or G) - TA(A or G) - TXC - GCX - GG (SEQ ID No. 18)

15   Primer E is sense primer and F is antisense primer.
Degeneracy is 128-fold.

Peptide 6: FLYXXTQIPHLAGTEQNFQLAK (SEQ ID No. 7)

20   PSM Primer "I" ACX - GA(A or G) - CA(A or G) - AA(T or C) - TT(T or C) - CA(A or G) - CT (SEQ ID No. 19)

PSM Primer "J" AG - (T or C)TG - (A or G)AA - (A or G)TT - (T or C)TG - (T or C)TC - XGT (SEQ ID No. 20)
25
PSM Primer "K" GA(A or G) - CA(A or G) - AA(T or C) - TT(T or C) CA(A or G) - CT (SEQ ID No. 21)

PSM Primer "L" AG - (T or C)TG - (A or G)AA - (A or G)TT -
30   (T or C)TG - (T or C)TC (SEQ ID No. 22)

Primers I and K are sense primers and J and L are anti-sense. I and J have degeneracies of 128-fold and K and L have 32-fold degeneracy.

35

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Peptide 7:   **TILFAS (W) DAEEFGXX (q) STE (e) A (E) ...** (SEQ ID No. 12)

PSM Primer "M"   TGG - GA(T or C) - GCX - GA(A or G) - GA(A
5   or G) - TT(C or T) - GG (SEQ ID No. 23)

PSM Primer "N"   CC - (G or A)AA - (T or C)TC - (T or C)TC - XGC - (A or G)TC - CCA (SEQ ID No. 24)

10   PSM Primer "O"   TGG - GA(T or C) - GCX - GA(A or G) - GA(A or G) - TT (SEQ ID No. 25)

PSM Primer "P"   AA - (T or C)TC - (T or C)TC - XGC - (A or G)TC - CCA (SEQ ID No. 26)

15

Primers M and O are sense primers and N and P are anti-sense. M and N have degeneracy of 64-fold and O and P are 32-fold degenerate.

20   Degenerate PCR was performed using a Perkin-Elmer Model 480 DNA thermal cycler. cDNA template for the PCR was prepared from LNCaP mRNA which had been isolated by standard methods of oligo dT chromatography (Collaborative Research). The cDNA synthesis was carried out as follows:

25

4.5µl   LNCaP poly A+ RNA (2µg)
1.0µl   Oligo dT primers (0.5µg)
4.5µl   dH₂O
10µl

30

Incubate at 68°C x 10 minutes.
Quick chill on ice x 5 minutes.

Add:

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4μl	5 x RT Buffer
2μl	0.1M DTT
1μl	10mM dNTPs
0.5μl	RNasin (Promega)
5	1.5μl	dH₂O
	19μl

Incubate for 2 minutes at 37°C.

Add 1μl Superscript® Reverse Transcriptase (Gibco®-BRL)

10   Incubate for 1 hour at 37°C.

Add 30μl dH₂O.

Use 2μl per PCR reaction.

15   Degenerate PCR reactions were optimized by varying the
annealing temperatures, Mg++ concentrations, primer
concentrations, buffer composition, extension times and
number of cycles. Our optimal thermal cycler profile was:
Denaturation at 94°C x 30 seconds, Annealing at 45-55°C for
20   1 minute (depending on the mean T_m of the primers used), and
Extension at 72°C for 2 minutes.

5μl	10 x PCR Buffer*
5μl	2.5mM dNTP Mix
25 5μl	Primer Mix (containing 0.5-1.0μg each of sense & anti-sense primers)
5μl	100mM ß-mercaptoethanol
2μl	LNCaP cDNA template
5μl	25mM MgCl₂ (2.5mM final)
30 21μl	dH₂O
2μl	diluted Taq Polymerase (0.5U/μl)

50μl total volume

Tubes were overlaid with 60μl of light mineral oil and

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amplified for 30 cycles. PCR products were analyzed by electrophoresing 5μl of each sample on a 2-3% agarose gel followed by staining with Ethidium bromide and photography.

5   *10x PCR Buffer
     166mM NH$_4$SO$_4$
     670mM Tris, pH 8.8
     2mg/ml BSA

10   Representative photographs displaying PCR products are shown in Figure 5.

Cloning of PCR Products

In order to further analyze these PCR products, these
15   products were cloned into a suitable plasmid vector using "TA Cloning" (Invitrogen® Corp.). The cloning strategy employed here is to directly ligate PCR products into a plasmid vector possessing overhanging T residues at the insertion site, exploiting the fact that Taq polymerase
20   leaves overhanging A residues at the ends of the PCR products. The ligation mixes are transformed into competent E. coli cells and resulting colonies are grown up, plasmid DNA is isolated by the alkaline lysis method (24), and screened by restriction analysis (Figure 6 A-B).
25

DNA Sequencing of PCR Products

TA Clones of PCR products were then sequenced by the dideoxy method (25) using Sequenase (U.S. Biochemical). 3-4μg of each plasmid DNA was denatured with NaOH and ethanol
30   precipitated. Labeling reactions were carried out as per the manufacturers recommendations using ^{35}S-ATP, and the reactions were terminated as per the same protocol. Sequencing products were then analyzed on 6% polyacrylamide/7M Urea gels using an IBI sequencing

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apparatus.   Gels were run at 120 watts for 2 hours.
Following electrophoresis, the gels were fixed for 15-20
minutes in 10% methanol/10% acetic acid, transferred onto
Whatman 3MM paper and dried down in a Biorad® vacuum dryer

5   at 80°C for 2 hours.   Gels were then autoradiographed at
room temperature for 16-24 hours.   In order to determine
whether the PCR products were the correct clones, we
analyzed the sequences obtained at the 5' and 3' ends of the
molecules looking for the correct primer sequences, as well

10   as adjacent sequences which corresponded to portions of the
peptides not used in the design of the primers.

IN-20 was confirmed to be correct and represent a partial
cDNA for the PSM gene.   In this PCR reaction, I and N

15   primers were used.   The DNA sequence we obtained when
reading from the I primer was:

ACG GAG CAA AAC TTT CAG CTT GCA AAG (SEQ ID No. 30)
    T   E   Q   N   F   Q   L   A   K (SEQ ID No. 31)

20

The underlined amino acids were the portion of peptide 6
that was used to design this sense primer and the remaining
amino acids which agree with those present within our
peptide confirm that this end of the molecule represents the

25   correct protein (PSM antigen).

When we analyzed the other end of the molecule by reading
from the N primer the sequence was:

30   **CTC TTC GGC ATC CCA GCT TGC AAA CAA AAT TGT TCT** (SEQ ID No.
32)

Since this represents the anti-sense DNA sequence, we need
to show the complementary sense sequence in order to find

35   our peptide.

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Sense Sequence:

AGA ACA ATT TTG TTT GCA AGC TGG GAT GCC AAG GAG (SEQ ID No. 33)

5   **R   T   I   L   F   A   S   W   D   A   E   E** (SEQ ID No. 34)

The underlined amino acids here represent the portion of peptide 7 used to create primer N. All of the amino acids
10   upstream of this primer are correct in the IN-20 clone, agreeing with the amino acids found in peptide 7. Further DNA sequencing has enabled us to identify the presence of our other PSM peptides within the DNA sequence of our positive clone.

15

The DNA sequence of this partial cDNA was found to be unique when screened on the Genbank computer database.

cDNA Library Construction and Cloning of Full - Length PSM
20   cDNA
A cDNA library from LNCaP mRNA was constructed using the Superscript® plasmid system (BRL®-Gibco). The library was transformed using competent DH5-α cells and plated onto 100mm plates containing LB plus 100μg/ml of Carbenicillin.
25   Plates were grown overnight at 37°C and colonies were transferred to nitrocellulose filters. Filters were processed and screened as per Grunstein and Hogness (26), using our 1.1kb partial cDNA homologous probe which was radiolabelled with ^{32}P-dCTP by random priming (27). We
30   obtained eight positive colonies which upon DNA restriction and sequencing analysis proved to represent full-length cDNA molecules coding for the PSM antigen. Shown in Figure 7 is an autoradiogram showing the size of the cDNA molecules represented in our library and in Figure 8 restriction

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analysis of several full-length clones is shown. Figure 9 is a plasmid Southern analysis of the samples in figure 8, showing that they all hybridize to the 1.1kb partial cDNA probe.

Both the cDNA as well as the antigen have been screened through the Genbank Computer database (Human Genome Project) and have been found to be unique.

Northern Analysis of PSM Gene Expression

Northern analysis (28) of the PSM gene has revealed that expression is limited to the prostate and to prostate carcinoma.

RNA samples (either 10μg of total RNA or 2μg of poly A+ RNA) were denatured and electrophoresed through 1.1% agarose/formaldehyde gels at 60 milliamps for 6-8 hours. RNA was then transferred to Nytran® nylon membranes (Schleicher and Schuell®) by pressure blotting in 10x SSC with a Posi-blotter (Stratagene®). RNA was cross-linked to the membranes using a Stratalinker (Stratagene®) and subsequently baked in a vacuum oven at 80°C for 2 hours. Blots were pre-hybridized at 65°C for 2 hours in prehybridization solution (BRL®) and subsequently hybridized for 16 hours in hybridization buffer (BRL®) containing 1-2 x 10^6 cpm/ml of ^{32}P-labelled random-primed cDNA pro$^{r}_{in}$ Membranes were washed twice in 1x SSPE/1% SDS and tw$_{and}$ 0.1x SSPE/1% SDS at 42°C. Membranes were then air-autoradiographed for 12-36 hours at -70°C.

PCR Analysis of PSM Gene Expression _j_ Tissues
PCR was performed on 15 human prost_
PSM gene expression. Five sample_

Δ Prostate
-s to determine
.t normal prostate

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tissue, benign prostatic hyperplasia, and prostate cancer were used (histology confirmed by MSKCC Pathology Department).

5   10μg of total RNA from each sample was reverse transcribed to made cDNA template as previously described in section IV. The primers used corresponded to the 5' and 3' ends of our 1.1kb partial cDNA, IN-20, and therefore the expected size of the amplified band is 1.1kb. Since the T_m of our primers

10   is 64°C we annealed the primers in our PCR at 60°C. We carried out the PCR for 35 cycles using the same conditions previously described in section IV.

LNCaP and H26 - Ras transfected LNCaP (29) were included as

15   a positive control and DU-145 as a negative control. 14/15 samples clearly amplified the 1.1kb band and therefore express the gene.

Experimental Results

20   The gene which encodes the 100kD PSM antigen has been identified. The complete cDNA sequence is shown in Sequence ID #1. Underneath that nucleic acid sequence is the predicted translated amino acid sequence. The total number of the amino acids is 750, ID #2. The hydrophilicity of the

25   predicted protein sequence is shown in Figure 16. Shown in Figure 17 are three peptides with the highest point of hydrophilicity. They are: Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID No. 35); Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No. 36; and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No. 37).

30

By the method of Klein, Kanehisa and DeLisi, a specific membrane-spanning domain is identified. The sequence is from the amino acid #19 to amino acid #44: Ala-Gly-Ala-Leu-Val-Leu-Aal-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID

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No. 38).

This predicted membrane-spanning domain was computed on PC Gene (computer software program). This data enables
5   prediction of inner and outer membrane domains of the PSM antigen which aids in designing antibodies for uses in targeting and imaging prostate cancer.

When the PSM antigen sequence with other known sequences of
10 the GeneBank were compared, homology between the PSM antigen sequence and the transferrin receptor sequence were found. The data are shown in Figure 18.

Experimental Discussions
15

Potential Uses for PSM Antigen:

    1.   Tumor detection:
Microscopic:
20 Unambiguous tumor designation can be accomplished by use of probes for different antigens. For prostatic cancer, the PSM antigen probe may prove beneficial. Thus PSM could be used for diagnostic purposes and this could be accomplished at the microscopic level using in-situ hybridization using
25 sense (control) and antisense probes derived from the coding region of the cDNA cloned by the applicants. This could be used in assessment of local extraprostatic extension, involvement of lymph node, bone or other metastatic sites. As bone metastasis presents a major problem in prostatic
30 cancer, early detection of metastatic spread is required especially for staging. In some tumors detection of tumor cells in bone marrow portends a grim prognosis and suggests that interventions aimed at metastasis be tried. Detection of PSM antigen expression in bone marrow aspirates or
35 sections may provide such early information.   PCR

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amplification or in-situ hybridization may be used. This
could be developed for any possible metastatic region.

        2.   Antigenic site identification
5   The knowledge of the cDNA for the antigen also provides for
    the identification of areas that would serve as good
    antigens for the development of antibodies for use against
    specific amino acid sequences of the antigen.   Such
    sequences may be at different regions such as outside,
10 membrane or inside of the PSM antigen. The development of
    these   specific   antibodies   would   provide   for
    immunohistochemical identification of the antigen.   These
    derived antibodies could then be developed for use,
    especially ones that work in paraffin fixed sections as well
15 as frozen section as they have the greatest utility for
    immunodiagnosis.

        3.   Restriction fragment length polymorphism and
              genomic DNA
20 Restriction fragment length polymorphisms (RFLPS) have
    proven to be useful in documenting the progression of
    genetic damage that occurs during tumor initiation and
    promotion.   It may be that RFLP analysis will demonstrate
    that changes in PSM sequence restriction mapping may provide
25 evidence of predisposition to risk or malignant potential or
    progression of the prostatic tumor.

    Depending on the chromosomal location of the PSM antile
    the PSM antigen gene may serve as a useful chr‑
30 location marker for chromosome analysis.

        4.   Serum   ' if the
                                                    che serum
    With the development of antigen specific ʳresence of
    antigen or selected antigen fragments
35 they may provide for a serum markᵣ

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metastatic disease and be useful individually or in combination with other prostate specific markers.

5.   Imaging

5   As the cDNA sequence implies that the antigen has the characteristics of a membrane spanning protein with the majority of the protein on the exofacial surface, antibodies, especially monoclonal antibodies to the peptide fragments exposed and specific to the tumor may provide for
10 tumor imaging local extension of metastatic tumor or residual tumor following prostatectomy or irradiation. The knowledge of the coding region permits the generation of monoclonal antibodies and these can be used in combination to provide for maximal imaging purposes. Because the
15 antigen shares a similarity with the transferrin receptor based on cDNA analysis (approximately 54%), it may be that there is a specific normal ligand for this antigen and that identification of the ligand(s) would provide another means of imaging.

20

6.   Isolation of ligands

The PSM antigen can be used to isolate the normal ligand(s) that bind to it. These ligand(s) depending on specificity may be used for targeting, or their serum levels may be
25 predictive of disease status. If it is found that the normal ligand for PSM is a carrier molecule then it may be that PSM could be used to bind to that ligand for therapy purposes (like an iron chelating substance) to help remove the ligand from the circulation. If the ligand promotes
30 tumor growth or metastasis then providing soluble PSM antigen would remove the ligand from binding the prostate. Knowledge of PSM antigen structure could lend to generation of small fragment that binds ligand which could serve the same purpose.

35

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7.   Therapeutic uses

a) Ligands. The knowledge that the cDNA structure of PSM
antigen shares structural homology with the transferrin
receptor (54% on the nucleic acid level) implies that there
5   may be an endogenous ligand for the receptor that may or may
not be transferrin-like. Transferrin is thought to be a
ligand that transports iron into the cell after binding to
the transferrin receptor. However, apotransferrin is being
reported to be a growth factor for some cells which express
10   the transferrin receptor (30). Whether transferrin is a
ligand for this antigen or some other ligand binds to this
ligand remains to be determined. If a ligand is identified
it may carry a specific substance such as a metal ion (iron
or zinc or other) into the tumor and thus serve as a means
15   to deliver toxic substances (radioactive or cytotoxic
chemical i.e. toxin like ricin or cytotoxic alkylating agent
or cytotoxic prodrug) to the tumor.

The main metastatic site for prostatic tumor is the bone.
20   The bone and bone stroma are rich in transferrin. Recent
studies suggest that this microenvironment is what provides
the right "soil" for prostatic metastasis in the bone (31).
It may be that this also promotes attachment as well, these
factors which reduce this ability may diminish prostatic
25   metastasis to the bone and prostatic metastatic growth in
the bone.

It was found that the ligand for the new antigen (thought to
be an oncogene and marker of malignant phenotype in breast
30   carcinoma) served to induce differentiation of breast cancer
cells and thus could serve as a treatment for rather than
promotor of the disease. It may be that ligand binding to
the right region of PSM whether with natural ligand or with
an antibody may serve a similar function.

35

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Antibodies against PSM antigen coupled with a cytotoxic agent will be useful to eliminate prostate cancer cells. Transferrin receptor antibodies with toxin conjugates are cytotoxic to a number of tumor cells as tumor cells tend to express increased levels of transferrin receptor (32). Transferrin receptors take up molecules into the cell by endocytosis. Antibody drug combinations can be toxic. Transferrin linked toxin can be toxic.

b) Antibodies against PSM antigen coupled with a cytotoxic agent will be useful to eliminate prostate cancer cells. The cytotoxic agent may be a radioisotope or toxin as known in ordinary skill of the art. The linkage of the antibody and the toxin or radioisotope can be chemical. Examples of direct linked toxins are doxorubicin, chlorambucil, ricin, pseudomonas exotoxin etc., or a hybrid toxin can be generated ½ with specificity for PSM and the other ½ with specificity for the toxin. Such a bivalent molecule can serve to bind to the tumor and the other ½ to deliver a cytotoxic to the tumor or to bind to and activate a cytotoxic lymphocyte such as binding to the T_1 - T_3 receptor complex. Antibodies of required specificity can also be cloned into T cells and by replacing the immunoglobulin domain of the T cell receptor (TcR); cloning in the desired MAb heavy and light chains; splicing the U_h and U_L gene segments with the constant regions of the α and ß TCR chains and transfecting these chimeric Ab/TcR genes in ƒ patients' T cells, propagating these hybrid cell of infusing them into the patient (33). Specific know Ab's tissue specific antigens for targets and generati Sable specific for such targets will help make t Ovides approach. Because the PSM antigen coding sible to used in knowledge of the entire coding region, generate a number of antibodies which

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combination to achieve an additive or synergistic anti-tumor
action. The antibodies can be linked to enzymes which can
activate non-toxic prodrugs at its site of the tumor such as
Ab-carboxypeptidase and 4-(bis(2 chloroethyl)amino)benzoyl-
5   α-glutamic acid and its active parent drug in mice (34).

It is possible to produce a toxic genetic chimera such as
TP-40 a genetic recombinant that possesses the cDNA from
TGF-alpha and the toxic portion of pseudomonas exotoxin so
10   the TGF and portion of the hybrid binds the epidermal growth
factor receptor (EGFR) and the pseudomonas portion gets
taken up into the cell enzymatically and inactivates the
ribosomes ability to perform protein synthesis resulting in
cell death. When we know the ligand for the PSM antigen we
15   can do the same.

In addition, once the ligand for the PSM antigen is
identified, toxin can be chemically conjugated to the
ligands. Such conjugated ligands can be therapeutically
20   useful.   Examples   of   the   toxins   are   daunomycin,
chlorambucil,   ricin,   pseudomonas   exotoxin,   etc.
Alternatively, chimeric construct can be created linking the
cDNA of the ligand with the cDNA of the toxin. An example
of such toxin is TGFα and pseudomonas exotoxin (35).
25

8.   Others
The PSM antigen may have other uses. It is well known that
the prostate is rich in zinc, if the antigen provides
function relative to this or other biologic function the PSM
30   antigen may provide for utility in the treatment of other
prostatic pathologies such as benign hyperplastic growth
and/or prostatitis.

Because purified PSM antigen can be generated, the purified
35   PSM antigen can be linked to beads and use it like a

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standard "affinity" purification. Serum, urine or other
biological samples can be used to incubate with the PSM
antigen bound onto beads.   The beads may be washed
thoroughly and then eluted with salt or pH gradient.   The
5   eluted material is SDS gel purified and used as a sample for
microsequencing. The sequences will be compared with other
known proteins and if unique, the technique of degenerated
PCR can be employed for obtaining the ligand. Once known,
the affinity of the ligand will be determined by standard
10   protocols (15).

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Second Series of Experiments

Expression of the Prostate-Specific Membrane Antigen

5   Applicant's have recently cloned a 2.65 kb complementary DNA
encoding PSM, the prostate-specific membrane antigen
recognized by the 7E11-C5.3 anti-prostate monoclonal
antibody. Immunohistochemical analysis of the LNCaP, DU-
145, and PC-3 prostate cancer cell lines for PSM expression
10   using the 7E11-C5.3 antibody reveals intense staining in the
LNCaP cells, with no detectable expression in both the DU-
145 and PC-3 cells. Coupled *in-vitro* transcription/
translation of the 2.65 kb full-length PSM cDNA yields an
84 kDa protein corresponding to the predicted polypeptide
15   molecular weight of PSM. Post-translational modification of
this protein with pancreatic canine microsomes yields the
expected 100 kDa PSM antigen. Following transfection of PC-
3 cells with the full-length PSM cDNA in a eukaryotic
expression vector applicant's detect expression of the PSM
20   glycoprotein by Western analysis using the 7E11-C5.3
monoclonal antibody. Ribonuclease protection analysis
demonstrates that the expression of PSM mRNA is almost
entirely prostate-specific in human tissues. PSM expression
appears to be highest in hormone-deprived states and is
25   hormonally modulated by steroids, with DHT downregulating
PSM expression in the human prostate cancer cell line LNCaP
by 8-10 fold, testosterone downregulating PSM by 3-4 fold,
and corticosteroids showing no significant effect. Normal
and malignant prostatic tissues consistently show high PSM
30   expression, whereas we have noted heterogeneous, and at
times absent, expression of PSM in benign prostatic
hyperplasia. LNCaP tumors implanted and grown both
orthotopically and subcutaneously in nude mice, abundantly
express PSM providing an excellent *in-vivo* model system to
35   study the regulation and modulation of PSM expression.

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Experimental D tails

Materials and Methods

5   Cells and Reagents:

The LNCaP, DU-145, and PC-3 cell lines were obtained from the American Type Culture Collection. Details regarding the establishment and characteristics of these cell lines have been previously published (5A,7A,8A). Unless specified

10   otherwise, LNCaP cells were grown in RPMI 1640 media supplemented with L-glutamine, nonessential amino acids, and 5% fetal calf serum (Gibco-BRL, Gaithersburg, MD.) in a CO_2 incubator at 37C. DU-145 and PC-3 cells were grown in minimal essential medium supplemented with 10% fetal calf

15   serum. All cell media were obtained from the MSKCC Media Preparation Facility. Restriction and modifying enzymes were purchased from Gibco-BRL unless otherwise specified.

Immunohistochemical Detection of PSM

20   We employed the avidin-biotin method of detection to analyze prostate cancer cell lines for PSM antigen expression (9A). Cell cytospins were made on glass slides using $5x10^4$ cells/100ul per slide. Slides were washed twice with PBS and then incubated with the appropriate suppressor serum for

25   20 minutes. The suppressor serum was drained off and the cells were incubated with diluted 7E11-C5.3 (5g/ml) monoclonal antibody for 1 hour. Samples were then washed with PBS and sequentially incubated with secondary antibodies for 30 minutes and with avidin-biotin complexes

30   for 30 minutes. Diaminobenzidine served as our chromogen and color development followed by hematoxylin counterstaining and mounting. Duplicate cell cytospins were used as controls for each experiment. As a positive control, the anti-cytokeratin monoclonal antibody CAM 5.2

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was used following the same procedure described above. Human EJ bladder carcinoma cells served as a negative control.

In-Vitro Transcription/Translation of PSM Antigen

Plasmid 55A containing the full length 2.65 kb PSM cDNA in the plasmid pSPORT 1 (Gibco-BRL) was transcribed in-vitro using the Promega TNT system (Promega Corp. Madison, WI). T7 RNA polymerase was added to the cDNA in a reaction mixture containing rabbit reticulocyte lysate, an amino acid mixture lacking methionine, buffer, and ^{35}S-Methionine (Amersham) and incubated at 30C for 90 minutes. Post-translational modification of the resulting protein was accomplished by the addition of pancreatic canine microsomes into the reaction mixture (Promega Corp. Madison, WI.). Protein products were analyzed by electrophoresis on 10% SDS-PAGE gels which were subsequently treated with Amplify autoradiography enhancer (Amersham, Arlington Heights, IL.) according to the manufacturers instructions and dried at 80C in a vacuum dryer. Gels were autoradiographed overnight at -70C using Hyperfilm MP (Amersham).

Transfection of PSM into PC-3 Cells

The full length PSM cDNA was subcloned into the pREP7 eukaryotic expression vector (Invitrogen, San Diego, CA.). Plasmid DNA was purified from transformed DH5-alpha bacteria (Gibco-BRL) using Qiagen maxi-prep plasmid isolation columns (Qiagen Inc., Chatsworth, CA.). Purified plasmid DNA (6-10g) was diluted with 900ul of Optimem media (Gibco-BRL) and mixed with 30ul of Lipofectin reagent (Gibco-BRL) which had been previously diluted with 900l of Optimem media. This mixture was added to T-75 flasks of 40-50% confluent PC-3 cells in Optimem media. After 24-36 hours, cells were trypsinized and split into 100mm dishes containing RPMI

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1640 media supplemented with 10% fetal calf serum and 1
mg/ml of Hygromycin B (Calbiochem, La Jolla, CA.). The dose
of Hygromycin B used was previously determined by a time
course/dose response cytotoxicity assay.   Cells were
5   maintained in this media for 2-3 weeks with changes of media
and Hygromycin B every 4-5 days until discrete colonies
appeared.   Colonies were isolated using 6mm cloning
cylinders and expanded in the same media. As a control, PC-
3 cells were also transfected with the pREP7 plasmid alone.
10   RNA was isolated from the transfected cells and PSM mRNA
expression was detected by both RNase Protection analysis
(described later) and by Northern analysis.

Western Blot Detection of PSM Expression

Crude protein lysates were isolated from LNCaP, PC-3, and
PSM-transfected PC-3 cells as previously described (10A).
5   LNCaP cell membranes were also isolated according to
published methods (10A).   Protein concentrations were
quantitated by the Bradford method using the BioRad protein
reagent kit (BioRad, Richmond, CA.). Following denaturation,
20g of protein was electrophoresed on a 10% SDS-PAGE gel at
10   25 mA for 4 hours.   Gels were electroblotted onto Immobilon
P membranes (Millipore, Bedford, MA.) overnight at 4C.
Membranes were blocked in 0.15M NaCl/0.01M Tris-HCl (TS)
plus 5% BSA followed by a 1 hour incubation with 7E11-C5.3
monoclonal antibody (10g/ml).   Blots were washed 4 times
15   with 0.15M NaCl/0.01M Tris-HCl/0.05% Triton-X 100 (TS-X) and
incubated for 1 hour with rabbit anti-mouse IgG (Accurate
Scientific, Westbury, N.Y.) at a concentration of 10g/ml.

Blots were then washed 4 times with TS-X and labeled with
20   ^{125}I-Protein A (Amersham, Arlington Heights, IL.) at a
concentration of 1 million cpm/ml. Blots were then washed
4 times with TS-X and dried on Whatman 3MM paper, followed

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by overnight autoradiography at -70C using Hyperfilm MP (Amersham).

Orthotopic and Subcutaneous LNCaP Tumor Growth in Nude Mice

LNCaP cells were harvested from sub-confluent cultures by a one minute exposure to a solution of 0.25% trypsin and 0.02% EDTA. Cells were resuspended in RPMI 1640 media with 5% fetal bovine serum, washed and diluted in either Matrigel (Collaborative Biomedical Products, Bedford, MA.) or calcium and magnesium-free Hank's balanced salt solution (HBSS). Only single cell suspensions with greater than 90% viability by trypan blue exclusion were used for in vivo injection. Male athymic Swiss (nu/nu) nude mice 4-6 weeks of age were obtained from the Memorial Sloan-Kettering Cancer Center Animal Facility. For subcutaneous tumor cell injection one million LNCaP cells resuspended in 0.2 mls. of Matrigel wer injected into the hindlimb of each mouse using a disposable syringe fitted with a 28 gauge needle. For orthotopic injection, mice were first anesthetized with an intraperitoneal injection of Pentobarbital and placed in the supine position. The abdomen was cleansed with Betadine and the prostate was exposed through a midline incision. 2.5 million LNCaP tumor cells in 0.1 ml. were injected directly into either posterior lobe using a 1 ml disposable syringe and a 28 gauge needle. LNCaP cells with and without Matrigel were injected. Abdominal closure was achieved in one layer using Autoclip wound clips (Clay Adams, Parsippany, N.J.).   Tumors were harvested in 6-8 weeks, confirmed histologically by faculty of the Memorial Sloan-Kettering Cancer Center Pathology Department, and frozen in liquid nitrogen for subsequent RNA isolation.

RNA Isolation

Total cellular RNA was isolated from cells and tissues by standard techniques (11,12) as well as by using RNAzol B

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(Cinna/Biotecx, Houston, TX.).   RNA concentrations and
quality were assessed by UV spectroscopy on a Beckman DU 640
spectrophotometer and by gel analysis.   Human tissue total
RNA samples were purchased from Clontech Laboratories, Inc.,
5   Palo Alto, CA.

Ribonuclease Protection Assays

A portion of the PSM cDNA was subcloned into the plasmid
vector pSPORT 1 (Gibco-BRL) and the orientation of the cDNA
10   insert relative to the flanking T7 and SP6 RNA polymerase
promoters   was   verified   by   restriction   analysis.
Linearization of this plasmid upstream of the PSM insert
followed by transcription with SP6 RNA polymerase yields a
400 nucleotide antisense RNA probe, of which 350 nucleotides
15   should be protected from RNase digestion by PSM RNA.   This
probe was used in figure 20.   Plasmid IN-20, containing a 1
kb partial PSM cDNA in the plasmid pCR II (Invitrogen) was
also used for riboprobe synthesis.   IN-20 linearized with
Xmn I (Gibco-BRL) yields a 298 nucleotide anti-sense RNA
20   probe when transcribed using SP6 RNA polymerase, of which
260 nucleotides should be protected from RNase digestion by
PSM mRNA.   This probe was used in figures 21 and 22. Probes
were synthesized using SP6 RNA polymerase (Gibco-BRL), rNTPs
(Gibco-BRL), RNAsin (Promega), and ^{32}P-rCTP (NEN, Wilmington,
25   DE.) according to published protocols (13).   Probes were
purified over NENSORB 20 purification columns (NEN) and
approximately 1 million cpm of purified, radiolabeled PSM
probe was mixed with 10g of each RNA and hybridized
overnight at 45C using buffers and reagents from the RPA II
30   kit (Ambion, Austin, TX).   Samples were processed as per
manufacturer's   instructions   and   analyzed   on   5%
polyacrilamide/7M urea denaturing gels using Seq ACRYL
reagents (ISS, Natick, MA.).   Gels were pre-heated to 55C
and run for approximately 1-2 hours at 25 watts.   Gels were

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then fixed for 30 minutes in 10% methanol/10% acetic acid, dried onto Whatman 3MM paper at 80C in a BioRad vacuum dryer and autoradiographed overnight with Hyperfilm MP (Amersham). Quantitation of PSM expression was determined by using a
5   scanning laser densitometer (LKB, Piscataway, NJ.).

Steroid Modulation Experiment
LNCaP cells (2 million) were plated onto T-75 flasks in RPMI 1640 media supplemented with 5% fetal calf serum and grown
10   24 hours until approximately 30-40% confluent. Flasks were then washed several times with phophate-buffered saline and RPMI medium supplemented with 5% charcoal-extracted serum was added. Cells were then grown for another 24 hours, at which time dihydrotesterone, testosterone, estradiol,
15   progesterone, and dexamethasone (Steraloids Inc., Wilton, NH.) were added at a final concentration of 2 nM. Cells were grown for another 24 hours and RNA was then harvested as previously described and PSM expression
analyzed by ribonuclease protection analysis.
20

Experimental Results

Immunohistochemical Detection of PSM:
25   Using the 7E11-C5.3 anti-PSM monoclonal antibody, PSM expression is clearly detectable in the LNCaP prostate cancer cell line, but not in the PC-3 and DU-145 cell lines (figure 17) in agreement with previously published results (4A). All normal and malignant prostatic tissues analyzed
30   stained positively for PSM expression (unpublished data).

In-Vitro Transcription/Translation of PSM Antigen:
As shown in figure 18, coupled in-vitro transcription/ translation of the 2.65 kb full-length PSM cDNA yields an 84
35   kDa protein species in agreement with the expected protein

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product from the 750 amino acid PSM open reading frame.
Following post-translational modification using pancreatic
canine microsomes we obtained a 100 kDa glycosylated
protein species consistent with the mature, native PSM
5   antigen.

**Detection of PSM Antigen in LNCaP Cell Membranes and
Transfected PC-3 Cells:**
PC-3 cells transfected with the full length PSM cDNA in the
10   pREP7 expression vector were assayed for expression of SM
mRNA by Northern analysis (data not shown). A clone with
high PSM mRNA expression was selected for PSM antigen
analysis by Western blotting using the 7E11-C5.3 antibody.
In figure 19, the 100 kDa PSM antigen is well expressed in
15   LNCaP cell lysate and membrane fractions, as well as in PSM-
transfected PC-3 cells but not in native PC-3 cells. This
detectable expression in the transfected PC-3 cells proves
that the previously cloned 2.65 kb PSM cDNA encodes the
antigen recognized by the 7E11-C5.3 anti-prostate monoclonal
20   antibody and that the antigen is being appropriately
glycosylated in the PC-3 cells, since the antibody
recognizes a carbohydrate-containing epitope on PSM.

PSM mRNA Expression:
25   Expression of PSM mRNA in normal human tissues was analyzed
using ribonuclease protection assays. Tissue expression of
PSM appears predominantly within the prostate, with very low
levels of expression detectable in human brain and salivary
gland (figure 20). No detectable PSM mRNA expression was
30   evident in non-prostatic human tissues when analyzed by
Northern analysis (data not shown). We have also noted on
occasion detectable PSM expression in normal human small
intestine tissue, however this mRNA expression is variable
depending upon the specific riboprobe used (data not shown).
35   All samples of normal human prostate and human prostatic

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adenocarcinoma assayed have revealed clearly detectable PSM expression, whereas we have noted generally decreased or absent expression of PSM in tissues exhibiting benign hyperplasia (figure 21). In human LNCaP tumors grown both
5 orthotopically and subcutaneously in nude mice we detected abundant PSM expression with or without the use of matrigel, which is required for the growth of subcutaneously implanted LNCaP cells (figure 21). PSM mRNA expression is distinctly modulated by the presence of steroids in physiologic doses
10 (figure 22). DHT downregulated expression by 8-10 fold after 24 hours and testosterone diminished PSM expression by 3-4 fold. Estradiol and progesterone also downregulated PSM expression in LNCaP cells, perhaps as a result of binding to the mutated androgen receptor known to exist in the LNCaP
15 cell. Overall, PSM expression is highest in the untreated LNCaP cells grown in steroid-depleted media, a situation that we propose simulates the hormone-deprived (castrate) state in-vivo. This experiment was repeated at steroid dosages ranging from 2-200 nM and at time points from 6
20 hours to 7 days with similar results; maximal downregulation of PSM mRNA was seen with DHT at 24 hours at doses of 2-20 nM.

Experimental Discussion
25 In order to better understand the biology of the human prostate in both normal and neoplastic states, we need to enhance our knowledge by studying the various proteins and other features that are unique to this important gland. Previous research has provided two valuable prostatic bio-
30 markers, PAP and PSA, both of which have had a significant impact on the diagnosis, treatment, and management of prostate malignancies. Our present work describing the preliminary characterization of the prostate-specific membrane antigen (PSM) reveals it to be a gene with many
35 interesting features. PSM is almost entirely prostate-

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specific as are PAP and PSA, and as such may enable further delineation of the unique functions and behavior of the prostate. The predicted sequence of the PSM protein (3) and its presence in the LNCaP cell membrane as determined by
5   Western blotting and immunohistochemistry, indicate that it is an integral membrane protein. Thus, PSM provides an attractive cell surface epitope for antibody-directed diagnostic imaging and cytotoxic targeting modalities (14). The ability to synthesize the PSM antigen *in-vitro* and to
10 produce tumor xenografts maintaining high levels of PSM expression provides us with a convenient and attractive model system to further study and characterize the regulation and modulation of PSM expression. Also, the high level of PSM expression in the LNCaP cells provides an
15 excellent *in-vitro* model system. Since PSM expression is hormonally-responsive to steroids and may be highly expressed in hormone-refractory disease (15), it is imperative to elucidate the potential role of PSM in the evolution of androgen-independent prostate cancer. The
20 detection of PSM mRNA expression in minute quantities in brain, salivary gland, and small intestine warrants further investigation, although these tissues were negative for expression of PSM antigen by immunohistochemistry using the 7E11-C5.3 antibody (16). In all of these tissues,
25 particularly small intestine, we detected mRNA expression using a probe corresponding to a region of the PSM cDNA near the 3' end, whereas we were unable to detect expression when using a 5' end PSM probe. These results may indicate that the PSM mRNA transcript undergoes alternative splicing in
30 different tissues. Previous protein studies have suggested that the 7E11-C5.3 antibody may actually detect two other slightly larger protein species in addition to the 100 kDa PSM antigen (17). These other protein species can be seen in the LNCaP lysate and membrane samples in figure 19.
35 Possible origins of these proteins include alternatively

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spliced PSM mRNA, other genes distinct from but closely related to PSM, or different post-translational modifications of the PSM protein. We are currently investigating these possibilities.

Applicnat's approach is based on prostate tissue specific promotor:enzyme or cytokine chimeras. We will examine promotor specific activation of prodrugs such as non toxic gancyclovir which is converted to a toxic metabolite by herpes simplex thymidine kinase or the prodrug 4-(bis(2chloroethyl)amino)benzoyl-1-glutamic acid to the benzoic acid mustard alkylating agent by the pseudomonas carboxy peptidase G2. As these drugs are activated by the enzyme (chimera) specifically in the tumor the active drug is released only locally in the tumor environment, destroying the surrounding tumor cells. We will also examine the promotor specific activation of cytokines such as IL-12, IL-2 or GM-CSF for activation and specific antitumor vaccination. Lastly the tissue specific promotor activation of cellular death genes may also prove to be useful in this area.

Gene Therapy Chimeras

The establishment of "chimeric DNA" for gene therapy requires the joining of different segments of DNA together to make a new DNA that has characteristics of both precursor DNA species involved in the linkage. In this proposal the two pieces being linked involve different functional aspects of DNA, the promotor region which allows for the reading of the DNA for the formation of mRNA will provide specificity and the DNA sequence coding for the mRNA will provide for therapeutic functional DNA.

DNA-Specified Enzyme or Cytokine mRNA:

When effective, antitumor drugs can cause the regression of

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very large amounts of tumor. The main requirements for antitumor drug activity is the requirement to achieve both a long enough time (t) and high enough concentration (c) (cxt) of exposure of the tumor to the toxic drug to assure
5   sufficient cell damage for cell death to occur. The drug also must be "active" and the toxicity for the tumor greater than for the hosts normal cells (22). The availability of the drug to the tumor depends on tumor blood flow and the drugs diffusion ability. Blood flow to the tumor does not
10   provide for selectivity as blood flow to many normal tissues is often as great or greater than that to the tumor. The majority of chemotherapeutic cytotoxic drugs are often as toxic to normal tissue as to tumor tissue. Dividing cells are often more sensitive than non-dividing normal cells, but
15   in many slow growing solid tumors such as prostatic cancer this does not provide for antitumor specificity (22).

Previously a means to increase tumor specificity of antitumor drugs was to utilize tumor associated enzymes to
20   activate nontoxic prodrugs to cytotoxic agents (19). A problem with this approach was that most of the enzymes found in tumors were not totally specific in their activity and similar substrate active enzymes or the same enzyme at only slightly lower amounts was found in other tissue and
25   thus normal tissues were still at risk for damage.

To provide absolute specificity and unique activity, viral, bacterial and fungal enzymes which have unique specificity for selected prodrugs were found which were not present in
30   human or other animal cells. Attempts to utilize enzymes such as herpes simplex thymidine kinase, bacterial cytosine deaminase and carboxypeptidase G-2 were linked to antibody targeting systems with modest success (19). Unfortunately, antibody targeted enzymes limit the number of enzymes
35   available per cell. Also, most antibodies do not have a

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high tumor target to normal tissue ratio thus normal tissues are still exposed reducing the specificity of these unique enzymes. Antibodies are large molecules that have poor diffusion properties and the addition of the enzymes molecular weight further reduces the antibodies diffusion.

Gene therapy could produce the best desired result if it could achieve the specific expression of a protein in the tumor and not normal tissue in order that a high local concentration of the enzyme be available for the production in the tumor environment of active drug (21).

Cytokines:

Applicant's research group has demonstrated that Applicant's can specifically and non-toxically "cure" an animal of an established tumor, in models of bladder or prostate cancer. The prostate cancer was the more difficult to cure especially if it was grown orthotopically in the prostate.

Our work demonstrated that tumors such as the bladder and prostate were not immunogenic, that is the administration of irradiated tumor cells to the animal prior to subsequent administration of non-irradiated tumor cells did not result in a reduction of either the number of tumor cells to produce a tumor nor did it reduce the growth rate of the tumor. But if the tumor was transfected with a retrovirus and secreted large concentrations of cytokines such as Il-2 then this could act as an antitumor vaccine and could also reduce the growth potential of an already established and growing tumor. IL-2 was the best, GM-CSF also had activity whereas a number of other cytokines were much less active. In clinical studies just using IL-2 for immunostimulation, very large concentrations had to be given which proved to be toxic. The key to the success of the cytokine gene modified tumor cell is that the cytokine is produced at the tumor

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site locally and is not toxic and that it stimulates immune recognition of the tumor and allows specific and non toxic recognition and destruction of the tumor. The exact mechanisms of how IL-2 production by the tumor cell
5   activates immune recognition is not fully understood, but one explanation is that it bypasses the need for cytokine production by helper T cells and directly stimulates tumor antigen activated cytotoxic CD8 cells. Activation of antigen presenting cells may also occur.
10

Tissue Promotor-Specific Chimera DNA Activation

Non-Prostatic Tumor Systems:
It has been observed in non-prostatic tumors that the use of
15 promotor specific activation can selectively lead to tissue specific gene expression of the transfected gene. In melanoma the use of the tyrosinase promotor which codes for the enzyme responsible for melanin expression produced over a 50 fold greater expression of the promotor driven reporter
20 gene expression in melanoma cells and not non melanoma cells. Similar specific activation was seen in the melanoma cells transfected when they were growing in mice. In that experiment no non-melanoma or melanocyte cell expressed the tyrosinase drive reporter gene product. The research group
25 at Welcome Laboratories have cloned and sequenced the promoter region of the gene coding for carcinoembryonic antigen (CEA). CEA is expressed on colon and colon carcinoma cells but specifically on metastatic cytosine deaminase which converts 5 flurorocytosine into 5
30 fluorouracil and observed a large increase in the ability to selectively kill CEA promotor driven colon tumor cells but non dividing not dividing normal liver cells. In vivo they observed that bystander tumor cells which were not transfected with the cytosine deaminase gene were also
35 killed, and that there was no toxicity to the host animal as

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the large tumors were regressing following treatment. Herpes simplex virus, (HSV), thymidine kinase similarly activates the prodrug gancyclovir to be toxic towards dividing cancer cells and HSV thymidine kinase has be n

5   shown to be specifically activatable by tissue specific promoters.

Prostatic Tumor Systems:

The therapeutic key to effective cancer therapy is to

10   achieve specificity and spare the patient toxicity. Gene therapy may provide a key part to specificity in that non-essential tissues such as the prostate and prostatic tumors produce tissue specific proteins, such as acid phosphatase (PAP), prostate specific antigen (PSA), and a gene which we

15   cloned, prostate-specific membrane antigen (PSM). Tissues such as the prostate contain selected tissue specific transcription factors which are responsible for binding to the promoter region of the DNA of these tissue specific mRNA. The promoter for PSA has been cloned and we are

20   investigating its use as a prostate specific promotor for prostatic tumor cells. Usually patients who are being treated for metastatic prostatic cancer have been put on androgen deprivation therapy which dramatically reduces the expression of mRNA for PSA. PSM on the other hand increases

25   in expression with hormone deprivation which-means it would be even more intensely expressed on patients being treated with hormone therapy. Preliminary work in collaboration with Dr. John Isaacs' Laboratory demonstrates that PSM is expressed when the human chromosome region containing the

30   human PSM gene is transferred to the rat tumor AT-6. AT-6 is a metastatic androgen independent tumor. The same chromosome transferred into non prostate derived tissues or tumors is not expressed and thus these cells could be used

as an animal model for these experiments. PSA, PSM positive
Human LNCaP cells will be used for testing in nude mice.

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Third Series of Experiments

Sensitive Detection of Prostatic Hematogenous Micrometastases Using PSA and PSM-Derived Primers in the Polymerase Chain Reaction

We have developed a PCR-based assay enabling sensitive detection of hematogenous micrometastases in patients with prostate cancer. We performed "nested PCR", amplifying mRNA sequences unique to prostate-specific antigen and to the prostate-specific membrane antigen, and have compared their respective results. Micrometastases were detected in 2/30 patients (6.7%) by PCR with PSA-derived primers, while PSM-derived primers detected tumor cells in 19/16 patients (63.3%). All 8 negative controls were negative with both PSA and PSM PCR. Assays were repeated to confirm results, and PCR products were verified by DNA sequencing and Southern analysis. Patients harboring circulating prostatic tumor cells as detected by PSM, and not by PSA-PCR included 4 patients previously treated with radical prostatectomy and with non-measurable serum PSA levels at the time of this assay. The significance of these findings with respect to future disease recurrence and progression will be investigated.

Improvement in the overall survival of patients with prostate cancer will depend upon earlier diagnosis. Localized disease, without evidence of extra-prostatic spread, is successfully treated with either radical prostatectomy or external beam radiation, with excellent long-term results (2,3). The major problem is that approximately two-thirds of men diagnosed with prostate cancer already have evidence of advanced extra-prostatic spread at the time of diagnosis, for which there is at present no cure (4). The use of clinical serum markers

such as prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) have enabled clinicians to detect prostatic carcinomas earlier and provide useful parameters to follow responses to therapy (5). Yet, despite the advent

5   of sensitive serum PSA assays, radionuclide bone scans, CT scans and other imaging modalities, we are still unable to detect the presence of micrometastatic cells prior to their establishment of solid metastases. Previous work has been done utilizing the polymerase chain reaction to amplify mRNA

10   sequences unique to breast, leukemia, and other malignant cells in the circulation and enable early detection of micrometastases (6,7). Recently, a PCR-based approach utilizing primers derived from the PSA DNA sequence was published (8). In this study 3/12 patients with advanced,

15   stage D prostate cancer had detectable hematogenous micrometastases.

We have recently identified and cloned a 2.65 kb cDNA encoding the 100 kDa prostate-specific membrane antigen

20   (PSM) recognized by the anti-prostate monoclonal antibody 7E11-C5.3 (9). PSM appears to be an integral membrane glycoprotein which is very highly expressed in prostatic tumors and metastases and is almost entirely prostate-specific (10). Many anaplastic tumors and bone metastases

25   have variable and at times no detectable expression of PSA, whereas these lesions appear to consistently express high levels of PSM. Prostatic tumor cells that escape from the prostate gland and enter the circulation are likely to have the potential to form metastases and are possibly the more

30   aggressive and possibly anaplastic cells, a population of cells that may not express high levels of PSA, but may retain high expression of PSM. We therefore chose to utilize DNA primers derived from the sequences of both PSA and PSM in a PCR assay to detect micrometastatic cells in

35   the peripheral circulation. Despite the high level of

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amplification and sensitivity of conventional RNA PCR, we have utilized a "nested" PCR approach in which we first amplify a target sequence, and subsequently use this PCR product as the template for another round of PCR
5   amplification with a new set of primers totally contained within the sequence of the previous product. This approach has enabled us to increase our level of detection from one prostatic tumor cell per 10,000 cells to better than one cell per ten million cells.
10

Experimental Details

Materials and Methods

15   Cells and Reagents:
LNCaP and MCF-7 cells were obtained from the American Type Culture Collection (Rockville, MD.). Details regarding the establishment and characteristics of these cell lines have been previously published (11,12). Cells were grown in RPMI
20   1640 media supplemented with L-glutamine, nonessential amino acids, obtained from the MSKCC Media Preparation Facility, and 5% fetal calf serum (Gibco-BRL, Gaithersburg, MD.) in a CO_2 incubator at 37C. All cell media was obtained from the MSKCC Media Preparation Facility. Routine chemical reagents
25   were of the highest grade possible and were obtained from Sigma Chemical Company, St. Louis, MO.

Patient Blood Specimens
All blood specimens used in this study were from patients
30   seen in the outpatient offices of urologists on staff at MSKCC. Two anti-coagulated (purple top) tubes per patient were obtained at the time of their regularly scheduled blood draws. Specimen procurement was conducted as per the approval of the MSKCC Institutional Review Board. Samples

were promptly brought to the laboratory for immediate processing. Serum PSA and PAP determinations were performed by standard techniques by the MSKCC Clinical Chemistry Laboratory. PSA determinations were performed using the

5   Tandem PSA assay (Hybritech, San Diego, CA.). The eight blood specimens used as negative controls were from 2 males with normal serum PSA values and biopsy-proven BPH, one healthy female, 3 healthy males, one patient with bladder cancer, and one patient with acute promyelocytic leukemia.

10

Blood Sample Processing/RNA Extraction

4 ml of whole anticoagulated venous blood was mixed with 3 ml of ice cold phosphate buffered saline and then carefully layered atop 8 ml of Ficoll (Pharmacia, Uppsala, Sweden) in

15   a 15-ml polystyrene tube. Tubes were centrifuged at 200 x g for 30 min. at 4C. Using a sterile pasteur pipette, the buffy coat layer (approx. 1 ml.) was carefully removed and rediluted up to 50 ml with ice cold phosphate buffered saline in a 50 ml polypropylene tube. This tube was then

20   centrifuged at 2000 x g for 30 min at 4C. The supernatant was carefully decanted and the pellet was allowed to drip dry. One ml of RNazol B was then added to the pellet and total RNA was isolated as per manufacturers directions (Cinna/Biotecx, Houston, TX.). RNA concentrations and

25   purity were determined by UV spectroscopy on a Beckman DU 640 spectrophotometer and by gelanalysis.

Determination of PCR Sensitivity

RNA was isolated from LNCaP cells and from mixtures of LNCaP

30   and MCF-7 cells at fixed ratios (i.e. 1:100, 1:1000, etc.) using RNAzol B. Nested PCR was then performed as described below with both PSA and PSM primers in order to determine the limit of detection for the assay. LNCaP:MCF-7 (1:100,000) cDNA was diluted with distilled water to obtain

35   concentrations of 1:1,000,000 and 1:10,000,000. MCF-7 cells

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were chosen because they have been previously tested and shown not to express PSM by PCR.

Polymerase Chain Reaction

5   The PSA outer primers used span portions of exons 4 and 5 to yield a 486 bp PCR product and enable differentiation between cDNA and possible contaminating genomic DNA amplification. The upstream primer sequence beginning at nucleotide 494 in PSA cDNA sequence is 5'-

10   TACCCACTGCATCAGGAACA-3' (SEQ. ID. No. 39) and the downstream primer at nucleotide 960 is 5'-CCTTGAAGCACACCATTACA-3' (SEQ. ID. No. 40). The PSA inner upstream primer (beginning at nucleotide 559) 5'-ACACAGGCCAGGTATTTCAG-3' (SEQ. ID. No. 41) and the downstream primer (at nucleotide 894) 5'-

15   GTCCAGCGTCCAGCACACAG-3' (SEQ. ID. No. 42) yield a 355 bp PCR product. All primers were synthesized by the MSKCC Microchemistry Core Facility. 5g of total RNA was reverse-transcribed into cDNA in a total volume of 201 using Superscript reverse transcriptase (Gibco-BRL) according to

20   the manufacturers recommendations. 11 of this cDNA served as the starting template for the outer primer PCR reaction. The 201 PCR mix included: 0.5U Taq polymerase (Promega Corp., Madison, WI.), Promega reaction buffer, 1.5mM $MgCl_2$, 200M dNTPs, and 1.0M of each primer. This mix was then

25   transferred to a Perkin Elmer 9600 DNA thermal cycler and incubated for 25 cycles. The PCR profile was as follows: 94C x 15 sec., 60C x 15 sec., and 72C for 45 sec. After 25 cycles, samples were placed on ice, and 11 of this reaction mix served as the template for another round of PCR using

30   the inner primers. The first set of tubes were returned to the thermal cycler for 25 additional cycles. PSM-PCR required the selection of primer pairs that also spanned an intron in order to be certain that cDNA and not genomic DNA were being amplified. Since the genomic DNA sequence of PSM

has not yet been determined, this involved trying different
primer pairs until a pair was found that produced the
expected size PCR product when cDNA was amplified, but with
no band produced from a genomic DNA template, indicating the
5 presence of a large intron. The PSM outer primers yield a
946 bp product and the inner primers a 434 bp product. The
PSM outer upstream primer used was 5'-
ATGGGTGTTTGGTGGTATTGACC-3' (SEQ. ID. No. 43) (beginning at
nucleotide 1401) and the downstream primer (at nucleotide
10 2348) was 5'-TGCTTGGAGCATAGATGACATGC-3' (SEQ. ID. No. 44)
The PSM inner upstream primer (at nucleotide 1581) was 5'-
ACTCCTTCAAGAGCGTGGCG-3' (SEQ. ID. No. 45) and the downstream
primer (at nucleotide 2015) was 5'-AACACCATCCCTCCTCGAACC-
3'(SEQ. ID. No. 46). cDNA used was the same as for the PSA
15 assay. The 501 PCR mix included: 1U Taq Polymerase
(Promega), 250M dNTPs, 10mM -mercaptoethanol, 2mM $MgCl_2$, and
51 of a 10x buffer mix containing: 166mM NH_4SO_4, 670mM Tris
pH 8.8, and 2 mg/ml of acetylated BSA. PCR was carried out
in a Perkin Elmer 480 DNA thermal cycler with the following
20 parameters: 94C x 4 minutes for 1 cycle, 94C x 30 sec., 58C
x 1 minute, and 72C x 1 minute for 25 cycles, followed by
72C x 10 minutes. Samples were then iced and 21 of this
reaction mix was used as the template for another 25 cycles
with a new reaction mix containing the inner PSM primers.
25 cDNA quality was verified by performing control reactions
using primers derived from -actin yielding a 446 bp PCR
product. The upstream primer used was 5'-
AGGCCAACCGCGAGAAGATGA-3' (SEQ. ID. No. 47) (exon 3) and the
downstream primer was 5'-ATGTCACACTGGGGAAGC-3' (SEQ. ID. No.
30 48) (exon 4). The entire PSA mix and 101 of each PSM
reaction mix were run on 1.5-2% agarose gels, stained with
ethidium bromide and photographed in an Eagle Eye Video
Imaging System (Stratagene, Torrey Pines, CA.). Assays were
repeated at least 3 times to verify results.

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Cloning and Sequencing of PCR Products

PCR products were cloned into the pCR II plasmid vector using the TA cloning system (Invitrogen). These plasmids were transformed into competent E. coli cells using standard
5   methods (13) and plasmid DNA was isolated using Magic Minipreps (Promega) and screened by restriction analysis. TA clones were then sequenced by the dideoxy method (14) using Sequenase (U.S. Biochemical). 3-4g of each plasmid was denatured with NaOH and ethanol precipitated. Labeling
10   reactions were carried out according to the manufacturers recommendations using ^{35}S-dATP (NEN), and the reactions were terminated as discussed in the same protocol. Sequencing products were then analyzed on 6% polyacrilamide/7M urea gels run at 120 watts for 2 hours. Gels were fixed for 20
15   minutes in 10% methanol/10% acetic acid, transferred to Whatman 3MM paper and dried down in a vacuum dryer for 2 hours at 80C. Gels were then autoradiographed at room temperature for 18 hours.

Southern Analysis
20
Ethidium-stained agarose gels of PCR products were soaked for 15 minutes in 0.2N HCl, followed by 30 minutes each in 0.5N NaOH/1.5M NaCl and 0.1M Tris pH 7.5/1.5M NaCl. Gels were then equilibrated for 10 minutes in 10x SSC (1.5M
25   NaCl/0.15M Sodium Citrate. DNA was transferred onto Nytran nylon membranes (Schleicher and Schuell) by pressure blotting in 10x SSC with a Posi-blotter (Stratagene). DNA was cross-linked to the membrane using a UV Stratalinker (Stratagene). Blots were pre-hybridized at 65C for 2 hours
30   and subsequently hybridized with denatured ^{32}P-labeled, random-primed cDNA probes (either PSM or PSA)(9,15). Blots were washed twice in 1x SSPE/0.5% SDS at 42C and twice in 0.1x SSPE/0.5% SDS at 50C for 20 minutes each. Membranes were air-dried and autoradiographed for 30 minutes to 1 hour

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at -70C with Kodak X-Omat film.

Experimental Results

Our technique of PCR amplification with nested primers
5   improved our level of detection of prostatic cells from
     approximately one prostatic cell per 10,000 MCF-7 cells to
     better than one cell per million MCF-7 cells, using either
     PSA or PSM-derived primers (figures 26 and 27). This
     represents a substantial improvement in our ability to
10   detect minimal disease. Characteristics of the 16 patients
     analyzed with respect to their clinical stage, treatment,
     serum PSA and PAP values, and results of our assay are shown
     in table 1. In total, PSA-PCR detected tumor cells in 2/30
     patients (6.7%), whereas PSM-PCR detected cells in 19/30
15   patients (63.3%). There were no patients positive for tumor
     cells by PSA and not by PSM, while PSM provided 8 positive
     patients not detected by PSA. Patients 10 and 11 in table
     1, both with very advanced hormone-refractory disease were
     detected by both PSA and PSM. Both of these patients have
20   died since the time these samples were obtained. Patients
     4, 7, and 12, all of whom were treated with radical
     prostatectomies for clinically localized disease, and all of
     whom have non-measurable serum PSA values 1-2 years
     postoperatively were positive for circulating prostatic
25   tumor cells by PSM-PCR, but negative by PSA-PCR. A
     representative ethidium stained gel photograph for PSM-PCR
     is shown in figure 28. Samples run in lane A represent PCR
     products generated from the outer primers and samples in
     lanes labeled B are products of inner primer pairs. The
30   corresponding PSM Southern blot autoradiograph is shown in
     figure 29. The sensitivity of the Southern blot analysis
     exceeded that of ethidium staining, as can be seen in
     several samples where the outer product is not visible on
     figure 28, but is detectable by Southern blotting as shown
35   in figure 29. In addition, sample 3 on figures 28 and 29

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(patient 6 in figure 30) appears to contain both outer and inner bands that are smaller than the corresponding bands in the other patients. DNA sequencing has confirmed that the nucleotide sequence of these bands matches that of PSM, with the exception of a small deletion. This may represent either an artifact of PCR, alternative splicing of PSM mRNA in this patient, or a PSM mutation. We have noted similar findings with other samples on several occasions (unpublished data). All samples sequenced and analyzed by Southern analysis have been confirmed as true positives for PSA and PSM.

Experimental Details

The ability to accurately stage patients with prostate cancer at the time of diagnosis is clearly of paramount importance in selecting appropriate therapy and in predicting long-term response to treatment, and potential cure. Pre-surgical staging presently consists of physical examination, serum PSA and PAP determinations, and numerous imaging modalities including transrectal ultrasonography, CT scanning, radionuclide bone scans, and even MRI scanning. No present modality, however, addresses the issue of hematogenous micrometastatic disease and the potential negative impact on prognosis that this may produce. Previous work has shown that only a fractional percentage of circulating tumor cells will inevitably go on to form a solid metastasis (16), however, the detection of and potential quantification of circulating tumor cell burden may prove valuable in more accurately staging disease. The long-term impact of hematogenous micrometastatic disease must be studied by comparing the clinical courses of patients found to have these cells in their circulation with patients of similar stage and treatment who test negatively.

The significantly higher level of detection of tumor cells

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with PSM as compared to PSA is not surprising to us, since we have noted more consistent expression of PSM in prostate carcinomas of all stages and grades as compared to variable expression of PSA in more poorly differentiated and
5   anaplastic prostate cancers. We were surprised to detect tumor cells in the three patients that had undergone radical prostatectomies with subsequent undetectable amounts of serum PSA. These patients would be considered to be surgical "cures" by standard criteria, yet they apparently
10   continue to harbor prostatic tumor cells.   It will be interesting to follow the clinical course of these patients as compared to others without PCR evidence of residual disease. We are presently analyzing larger numbers of patient samples in order to verify these findings and
15   perhaps identify patients at risk for metastatic disease.

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Fourth Series of Experiments

**EXPRESSION OF THE PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSM)
DIMINISHES THE MITOGENIC STIMULATION OF AGGRESSIVE HUMAN
5   PROSTATIC CARCINOMA CELLS BY TRANSFERRIN**

An association between transferrin and human prostate cancer
has been suggested by several investigators. It has been
10   shown that the expressed prostatic secretions of patients
with prostate cancer are enriched with respect to their
content of transferrin and that prostate cancer cells are
rich in transferrin receptors (J. Urol. 143, 381, 1990).
Transferrin derived from bone marrow has been shown to
15   selectively stimulate the growth of aggressive prostate
cancer cells (PNAS 89, 6197, 1992). We have previously
reported the cloning of the cDNA encoding the 100 kDa PSM
antigen (Cancer Res. 53, 208, 1993). DNA sequence analysis
has revealed that a portion of the coding region, from
20   nucleotide 1250 to 1700 possesses a 54% homology to the
human transferrin receptor. PC-3 cells do not express PSM
mRNA or protein and exhibit increased cell growth in
response to transferrin, whereas, LNCaP prostate cancer
cells which highly express PSM have a very weak response to
25   transferrin.   To determine whether PSM expression by
prostatic cancer cells impacts upon their mitogenic response
to transferrin we stably transfected the full-length PSM
cDNA into the PC-3 prostate cancer cells. Clones highly

expressing PSM mRNA were identified by Northern analysis and expression of PSM protein was verified by Western analysis using the anti-PSM monoclonal antibody 7E11-C5.3.

5   We plated $2x10^4$ PC-3 or PSM-transfected PC-3 cells per well in RPMI medium supplemented with 10% fetal bovine serum and at 24 hrs. added 1 μg per ml. of holotransferrin to the cells. Cells were counted at 1 day to be highly mitogenic to the PC-3 cells. Cells were counted at 1 day to determine
10   plating efficiency and at 5 days to determine the effect of the transferrin. Experiments were repeated to verify the results.

We found that the PC-3 cells experienced an average increase
15   of 275% over controls, whereas the LNCaP cells were only stimulated 43%. Growth kinetics revealed that the PSM-transfected PC-3 cells grew 30% slower than native PC-3 cells. This data suggests that PSM expression in aggressive, metastatic human prostate cancer cells
20   significantly abrogates their mitogenic response to transferrin.

The use of therapeutic vaccines consisting of cytokine-secreting tumor cell preparations for the treatment cf
25   established prostate cancer was investigated in the Dunning R3327-MatLyLu rat prostatic adenocarcinoma model. Only IL-2

secreting, irradiated tumor cell preparations were capable
of curing animals from subcutaneously established tumors,
and engendered immunological memory that protected the
animals from another tumor challenge. Immunotherapy was

5 less effective when tumors were induced orthotopically, but
nevertheless led to improved outcome, significantly
delaying, and occasionally preventing recurrence of tumors
after resection of the cancerous prostate. Induction of a
potent immune response in tumor bearing animals against the

10 nonimmunogenic MatLyLu tumor supports the view that active
immunotherapy of prostate cancer may have therapeutic
benefits.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Israeli, Ron S.
                Heston, Warren D.W.
                Fair, William R.

    (ii) TITLE OF INVENTION: PROSTATE-SPECIFIC MEMBRANE ANTIGEN

   (iii) NUMBER OF SEQUENCES: 38

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Cooper & Dunham
        (B) STREET: 30 Rockefeller Plaza
        (C) CITY: New York
        (D) STATE: New York
        (E) COUNTRY: United States of America
        (F) ZIP: 10112

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

  (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: White, John P.
        (B) REGISTRATION NUMBER: 28,678
        (C) REFERENCE/DOCKET NUMBER: 1747/41426

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (212) 977-9550
        (B) TELEFAX: (212) 664-0525
        (C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 2653 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (F) TISSUE TYPE: Carcinoma

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(vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate-Specific Membrane Antigen

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 262..2511

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTCAAAAGGG GCCGGATTTC CTTCTCCTGG AGGCAGATGT TGCCTCTCTC TCTCGCTCGG      60

ATTGGTTCAG TGCACTCTAG AAACACTGCT GTGGTGGAGA AACTGGACCC CAGGTCTGGA     120

GCGAATTCCA GCCTGCAGGG CTGATAAGCG AGGCATTAGT GAGATTGAGA GAGACTTTAC     180

CCCGCCGTGG TGGTTGGAGG GCGCGCAGTA GAGCAGCAGC ACAGGCGCGG GTCCCGGGAG     240

GCCGGCTCTG CTCGCGCCGA G ATG TGG AAT CTC CTT CAC GAA ACC GAC TCG      291
                        Met Trp Asn Leu Leu His Glu Thr Asp Ser
                         1                   5                  10

GCT GTG GCC ACC GCG CGC CGC CCG CGC TGG CTG TGC GCT GGG GCG CTG      339
Ala Val Ala Thr Ala Arg Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu
             15                  20                  25

GTG CTG GCG GGT GGC TTC TTT CTC CTC GGC TTC CTC TTC GGG TGG TTT      387
Val Leu Ala Gly Gly Phe Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe
             30                  35                  40

ATA AAA TCC TCC AAT GAA GCT ACT AAC ATT ACT CCA AAG CAT AAT ATG      435
Ile Lys Ser Ser Asn Glu Ala Thr Asn Ile Thr Pro Lys His Asn Met
             45                  50                  55

AAA GCA TTT TTG GAT GAA TTG AAA GCT GAG AAC ATC AAG AAG TTC TTA      483
Lys Ala Phe Leu Asp Glu Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu
         60                  65                  70

TAT AAT TTT ACA CAG ATA CCA CAT TTA GCA GGA ACA GAA CAA AAC TTT      531
Tyr Asn Phe Thr Gln Ile Pro His Leu Ala Gly Thr Glu Gln Asn Phe
 75                  80                  85                  90

CAG CTT GCA AAG CAA ATT CAA TCC CAG TGG AAA GAA TTT GGC CTG GAT      579
Gln Leu Ala Lys Gln Ile Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp
             95                 100                 105

TCT GTT GAG CTA GCA CAT TAT GAT GTC CTG TTG TCC TAC CCA AAT AAG      627
Ser Val Glu Leu Ala His Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys
             110                 115                 120

ACT CAT CCC AAC TAC ATC TCA ATA ATT AAT GAA GAT GGA AAT GAG ATT      675
Thr His Pro Asn Tyr Ile Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile
             125                 130                 135

TTC AAC ACA TCA TTA TTT GAA CCA CCT CCT CCA GGA TAT GAA AAT GTT      723
Phe Asn Thr Ser Leu Phe Glu Pro Pro Pro Gly Tyr Glu Asn Val
             140                 145                 150

TCG GAT ATT GTA CCA CCT TTC AGT GCT TTC TCT CCT CAA GGA ATG CCA      771
Ser Asp Ile Val Pro Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro
155                 160                 165                 170
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GAG GGC GAT CTA GTG TAT GTT AAC TAT GCA CGA ACT GAA GAC TTC TTT          819
Glu Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe
            175                 180                 185

AAA TTG GAA CGG GAC ATG AAA ATC AAT TGC TCT GGG AAA ATT GTA ATT          867
Lys Leu Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile
            190                 195                 200

GCC AGA TAT GGG AAA GTT TTC AGA GGA AAT AAG GTT AAA AAT GCC CAG          915
Ala Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln
            205                 210                 215

CTG GCA GGG GCC AAA GGA GTC ATT CTC TAC TCC GAC CCT GCT GAC TAC          963
Leu Ala Gly Ala Lys Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr
            220                 225                 230

TTT GCT CCT GGG GTG AAG TCC TAT CCA GAT GGT TGG AAT CTT CCT GGA         1011
Phe Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly
235                 240                 245                 250

GGT GGT GTC CAG CGT GGA AAT ATC CTA AAT CTG AAT GGT GCA GGA GAC         1059
Gly Gly Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp
            255                 260                 265

CCT CTC ACA CCA GGT TAC CCA GCA AAT GAA TAT GCT TAT AGG CGT GGA         1107
Pro Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly
            270                 275                 280

ATT GCA GAG GCT GTT GGT CTT CCA AGT ATT CCT GTT CAT CCA ATT GGA         1155
Ile Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly
            285                 290                 295

TAC TAT GAT GCA CAG AAG CTC CTA GAA AAA ATG GGT GGC TCA GCA CCA         1203
Tyr Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Pro
            300                 305                 310

CCA GAT AGC AGC TGG AGA GGA AGT CTC AAA GTG CCC TAC AAT GTT GGA         1251
Pro Asp Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn Val Gly
315                 320                 325                 330

CCT GGC TTT ACT GGA AAC TTT TCT ACA CAA AAA GTC AAG ATG CAC ATC         1299
Pro Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile
            335                 340                 345

CAC TCT ACC AAT GAA GTG ACA AGA ATT TAC AAT GTG ATA GGT ACT CTC         1347
His Ser Thr Asn Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu
            350                 355                 360

AGA GGA GCA GTG GAA CCA GAC AGA TAT GTC ATT CTG GGA GGT CAC CGG         1395
Arg Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg
            365                 370                 375

GAC TCA TGG GTG TTT GGT GGT ATT GAC CCT CAG AGT GGA GCA GCT GTT         1443
Asp Ser Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val
            380                 385                 390

GTT CAT GAA ATT GTG AGG AGC TTT GGA ACA CTG AAA AAG GAA GGG TGG         1491
Val His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp
395                 400                 405                 410

AGA CCT AGA AGA ACA ATT TTG TTT GCA AGC TGG GAT GCA GAA GAA TTT         1539
Arg Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe
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              415                   420                   425
GGT CTT CTT GGT TCT ACT GAG TGG GCA GAG GAG AAT TCA AGA CTC CTT      1587
Gly Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu
            430                   435                   440

CAA GAG CGT GGC GTG GCT TAT ATT AAT GCT GAC TCA TCT ATA GAA GGA      1635
Gln Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly
            445                   450                   455

AAC TAC ACT CTG AGA GTT GAT TGT ACA CCG CTG ATG TAC AGC TTG GTA      1683
Asn Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val
            460                   465                   470

CAC AAC CTA ACA AAA GAG CTG AAA AGC CCT GAT GAA GGC TTT GAA GGC      1731
His Asn Leu Thr Lys Glu Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly
475                   480                   485                   490

AAA TCT CTT TAT GAA AGT TGG ACT AAA AAA AGT CCT TCC CCA GAG TTC      1779
Lys Ser Leu Tyr Glu Ser Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe
                495                   500                   505

AGT GGC ATG CCC AGG ATA AGC AAA TTG GGA TCT GGA AAT GAT TTT GAG      1827
Ser Gly Met Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu
            510                   515                   520

GTG TTC TTC CAA CGA CTT GGA ATT GCT TCA GGC AGA GCA CGG TAT ACT      1875
Val Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr
            525                   530                   535

AAA AAT TGG GAA ACA AAC AAA TTC AGC GGC TAT CCA CTG TAT CAC AGT      1923
Lys Asn Trp Glu Thr Asn Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser
            540                   545                   550

GTC TAT GAA ACA TAT GAG TTG GTG GAA AAG TTT TAT GAT CCA ATG TTT      1971
Val Tyr Glu Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro Met Phe
555                   560                   565                   570

AAA TAT CAC CTC ACT GTG GCC CAG GTT CGA GGA GGG ATG GTG TTT GAG      2019
Lys Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Met Val Phe Glu
            575                   580                   585

CTA GCC AAT TCC ATA GTG CTC CCT TTT GAT TGT CGA GAT TAT GCT GTA      2067
Leu Ala Asn Ser Ile Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val
            590                   595                   600

GTT TTA AGA AAG TAT GCT GAC AAA ATC TAC AGT ATT TCT ATG AAA CAT      2115
Val Leu Arg Lys Tyr Ala Asp Lys Ile Tyr Ser Ile Ser Met Lys His
            605                   610                   615

CCA CAG GAA ATG AAG ACA TAC AGT GTA TCA TTT GAT TCA CTT TTT TCT      2163
Pro Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser
            620                   625                   630

GCA GTA AAG AAT TTT ACA GAA ATT GCT TCC AAG TTC AGT GAG AGA CTC      2211
Ala Val Lys Asn Phe Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu
635                   640                   645                   650

CAG GAC TTT GAC AAA AGC AAC CCA ATA GTA TTA AGA ATG ATG AAT GAT      2259
Gln Asp Phe Asp Lys Ser Asn Pro Ile Val Leu Arg Met Met Asn Asp
            655                   660                   665
```

```
CAA CTC ATG TTT CTG GAA AGA GCA TTT ATT GAT CCA TTA GGG TTA CCA    2307
Gln Leu Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro
            670             675             680

GAC AGG CCT TTT TAT AGG CAT GTC ATC TAT GCT CCA AGC AGC CAC AAC    2355
Asp Arg Pro Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn
            685             690             695

AAG TAT GCA GGG GAG TCA TTC CCA GGA ATT TAT GAT GCT CTG TTT GAT    2403
Lys Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp
        700             705             710

ATT GAA AGC AAA GTG GAC CCT TCC AAG GCC TGG GGA GAA GTG AAG AGA    2451
Ile Glu Ser Lys Val Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg
715             720             725             730

CAG ATT TAT GTT GCA GCC TTC ACA GTG CAG GCA GCT GCA GAG ACT TTG    2499
Gln Ile Tyr Val Ala Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu
                735             740             745

AGT GAA GTA GCC TAAGAGGATT CTTTAGAGAA TCCGTATTGA ATTTGTGTGG        2551
Ser Glu Val Ala
            750

TATGTCACTC AGAAAGAATC GTAATGGGTA TATTGATAAA TTTTAAAATT GGTATATTTG  2611

AAATAAAGTT GAATATTATA TATAAAAAAA AAAAAAAAAA AA                     2653
```

(2) INFORMATION FOR SEQ ID NO:2:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 750 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: protein

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Trp Asn Leu Leu His Glu Thr Asp Ser Ala Val Ala Thr Ala Arg
1               5               10              15

Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val Leu Ala Gly Gly Phe
            20              25              30

Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile Lys Ser Ser Asn Glu
            35              40              45

Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys Ala Phe Leu Asp Glu
    50              55              60

Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Gln Ile
65              70              75              80

Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu Ala Lys Gln Ile
                85              90              95

Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Ala His
            100             105             110

Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile
```

```
                 115                    120                    125
     Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe
         130                    135                    140

     Glu Pro Pro Pro Pro Gly Tyr Glu Asn Val Ser Asp Ile Val Pro Pro
     145                    150                    155                    160

     Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr
                     165                    170                    175

     Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met
                 180                    185                    190

     Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys Val
                 195                    200                    205

     Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Lys Gly
         210                    215                    220

     Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val Lys
     225                    230                    235                    240

     Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg Gly
                     245                    250                    255

     Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr
                 260                    265                    270

     Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly
         275                    280                    285

     Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys
         290                    295                    300

     Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Arg
     305                    310                    315                    320

     Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn
                     325                    330                    335

     Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser Thr Asn Glu Val
                 340                    345                    350

     Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro
                 355                    360                    365

     Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly
         370                    375                    380

     Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg
     385                    390                    395                    400

     Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile
                     405                    410                    415

     Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr
                     420                    425                    430

     Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala
                 435                    440                    445
```

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```
Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val
    450                 455                 ·460

Asp Cys Thr Pro Leu Met Tyr Ser Leu Val His Asn Leu Thr Lys Glu
465                 470                 475                 480

Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser
                485                 490                 495

Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile
                500                 505                 510

Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu
                515                 520                 525

Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn
                530                 535                 540

Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu
545                 550                 555                 560

Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His Leu Thr Val
                565                 570                 575

Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val
                580                 585                 590

Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala
                595                 600                 605

Asp Lys Ile Tyr Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr
610                 615                 620

Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr
625                 630                 635                 640

Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser
                645                 650                 655

Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu
                660                 665                 670

Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg
                675                 680                 685

His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser
    690                 695                 700

Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp
705                 710                 715                 720

Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala Ala
                725                 730                 735

Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala
                740                 745                 750
```

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid

```
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
           (A) ORGANISM: Homo sapiens
           (F) TISSUE TYPE: Carcinoma

     (vii) IMMEDIATE SOURCE:
           (B) CLONE: Prostate Specific Membrane Antigen


      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

       Ser Leu Tyr Glu Ser Xaa Thr Lys
       1               5

 (2) INFORMATION FOR SEQ ID NO:4:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 15 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
           (A) ORGANISM: Homo sapiens
           (F) TISSUE TYPE: Carcinoma

     (vii) IMMEDIATE SOURCE:
           (B) CLONE: Prostate Specific Membrane Antigen


      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

       Ser Tyr Pro Asp Gly Xaa Asn Leu Pro Gly Gly Gly Val Gln Arg
       1               5                   10                  15

 (2) INFORMATION FOR SEQ ID NO:5:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 7 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO
```

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        (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

       (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        Phe Tyr Asp Pro Met Phe Lys
        1   5

(2) INFORMATION FOR SEQ ID NO:6:

         (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 9 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

       (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

       (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

        Ile Tyr Asn Val Ile Gly Thr Leu Lys
        1   5

(2) INFORMATION FOR SEQ ID NO:7:

         (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 22 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

       (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

       (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Phe Leu Tyr Xaa Xaa Thr Gln Ile Pro His Leu Ala Gly Thr Glu Gln
1   5   10   15

Asn Phe Gln Leu Ala Lys
              20

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

  (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

  (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Asp Val
1   5   10   15

Lys

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

  (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

  (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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```
Pro Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val
1                 5                 10                15

Lys
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg
1                 5                 10                15
```

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 19 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile
1                 5                 10                15

Glu Ser Lys
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Xaa Xaa Glu
1               5                   10                  15

Ser Thr Glu Glu Ala Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
TTYTAYGAYC CNATGTT                                              17
```

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double

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```
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

     (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen


      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
```

AACATNGGRT CRTARAA   17

(2) INFORMATION FOR SEQ ID NO:15:

```
       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

     (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen


      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
```

ATHTAYAAYG TNATHGG   17

(2) INFORMATION FOR SEQ ID NO:16:

```
       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
```

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     (F) TISSUE TYPE: Carcinoma

(vii) IMMEDIATE SOURCE:
     (B) CLONE: Prostate Specific Membrane Antigen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCDATNACRT TRTADAT   17

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 17 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo sapien
      (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
      (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCNGCNGAYT AYTTYGC   17

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 17 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo sapien
      (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
      (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCRAARTART CNGCNGG   17

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(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACNGARCARA AYTTYCARCT   20

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGYTGRAART TYTGYTCNGT   20

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

   (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GARCARAAYT TYCARCT   17

(2) INFORMATION FOR SEQ ID NO:22:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: cDNA

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

       (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

      (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGYTGRAART TYTGYTC   17

(2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 20 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: cDNA

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

       (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapien
            (F) TISSUE TYPE: Carcinoma

      (vii) IMMEDIATE SOURCE:
            (B) CLONE: Prostate Specific Membrane Antigen

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TGGGAYGCNG ARGARTTYGG   20

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCRAAYTCYT CNGCRTCCCA   20

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapien
        (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
        (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGGGAYGCNG ARGARTT   17

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 17 base pairs
        (B) TYPE: nucleic acid

```
            (C)  STRANDEDNESS: double
            (D)  TOPOLOGY: linear

     (ii)  MOLECULE TYPE: cDNA

    (iii)  HYPOTHETICAL: NO

     (iv)  ANTI-SENSE: NO

     (vi)  ORIGINAL SOURCE:
            (A)  ORGANISM: Homo sapien
            (F)  TISSUE TYPE: Carcinoma

    (vii)  IMMEDIATE SOURCE:
            (B)  CLONE: Prostate Specific Membrane Antigen


     (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAYTCYTCNG CRTCCCA                                              17

(2) INFORMATION FOR SEQ ID NO:27:

      (i)  SEQUENCE CHARACTERISTICS:
            (A)  LENGTH: 780 base pairs
            (B)  TYPE: nucleic acid
            (C)  STRANDEDNESS: double
            (D)  TOPOLOGY: linear

     (ii)  MOLECULE TYPE: cDNA

    (iii)  HYPOTHETICAL: NO

     (iv)  ANTI-SENSE: NO


     (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:27:

TACACTTATC CCATTCGGAC ATGCCCACCT TGGAACTGGA GACCCTTACA CCCCAGGCTT    60

CCCTTCGTTC AACCACACCC ANNNGTTTCC ACCAGTTGAA TCTTCAGGAC TACCCCACAT   120

TGCTGTTCAG ACCATCTCTA GCAGTGCAGC AGCCAGGCTG TTCAGCAAAA TGGATGGAGA   180

CACATGCTCT GANAGNNGTT GGAAAGGTGC GATCCANNNT TCCTGTAAGG TNNGACNNAA   240

CAAAGCAGGA GANNNNGCCA GANTAATGGT GAAACTAGAT GTGAACAATT CCATGAAAGA   300

CAGGAAGATT CTGAACATCT TCGGTGCTAT CCAGGGATTT GAAGAACCTG ATCGGTATGT   360

TGTGATTGGA GCCCAGAGAG ACTCCTGGGG CCCAGGAGTG GCTAAAGCTG GCACTGGAAC   420

TGCTATATTG TTGGAACTTG CCCGTGTGAT CTCAGACATA GTGAAAAACG AGGGCTACAA   480

ACCGAGGCGA AGCATCATCT TTGCTAGCTG GAGTGCAGGA GACTACGGAG CTGTGGGTGC   540

TACTGAATGG CTGGAGGGGT ACTCTGCCAT GCTGCATGCC AAAGCTTTCA CTTACATCAN   600

NGCTTGGATG CTCCAGTCCT GGGAGCAAGC CATGTCAAGA TTTCTGCCAG CCCCTTGCTG   660

TATATGCTGC TGGGGAGTAT TATGAAGGGG GTGAAGAATC CAGCAGCAGT CTCAGAGAGC   720
```

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NNNNCTCTAT AACAGACTTG GCCCAGACTG GGTAAAAGCA GTTGTTCCTC TTGGCCTGGA   780

(2) INFORMATION FOR SEQ ID NO:28:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 660 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TGCAGAAAAG CTATTCAAAA ACATGGAAGG AAACTGTCCT CCTAGTTGGA ATATAGATTC   60

CTCATGTAAG CTGGAACTTT CACAGAATCA AAATGTGAAG CTCACTGTGA ACAATGTACT   120

GAAAGAAACA AGAATACTTA ACATCTTTGG CGTTATTAAA GGCTATGAGG AACCAGACCG   180

CTACATTGTA GTAGGAGCCC AGAGAGACGC TTGGGGCCCT GGTNGTTGCG AAGTCCAGTG   240

TGGGAACAGG TCTTNCTGTT GAAACTTGCC CAAGTATTCT CAGATATGAT TTCAAAAGAT   300

GGATTTAGAC CCAGCAGGAG TATTATCTTT GCCAGCTGGA CTGCAGGAGA CTATGGAGCT   360

GTTGGTCCGA CTGAGTGGCT GGAGGGGTAC CTTTCATCTT TGCATCTAAA GNNNGCTTTC   420

ACTTACATTA ATNCTGGATA AAGTCGTCCT GGGTACTAGC AACTTCAAGG TTTCTGCCAG   480

CCCCCTATTA TATACACTTA TGGGGAAGAT AATGCAGGAN NCGTAAAGCA TCCGANNNNN   540

NNNTTGATGG AAAATATCTA TATCGAAACA GTAATTGGAT TAGCAAAATT GAGGAACTTT   600

CCTTGGACAA TGCTGCATTC CCTTTTCTTG CATATTCAGG AATCCCAGCA GTTTCTTTCT   660

(2) INFORMATION FOR SEQ ID NO:29:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 540 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
TATGGAAGGA GACTGTCCCT CTGACTGGAA AACAGACTCT ACATGTAGGA TGGTAACCTC    60

AGAAAGCAAG AATGTGAAGC TCACTGTGAG CAATGTGCTG AAAGAGATAA AAATTCTTAA   120

CATCTTTGGA GTTATTAAAG GCTTTGTAGA ACCAGATCAC TATGTTGTAG TTGGGGCCCA   180

GAGAGATGCA TGGGGCCCTG GAGCTGCAAA ATCNCGGTGT AGGCACAGCT CTCCTATTGA   240

AACTTGCCCA GATGTTCTCA GATATGGTCT TAAAAGATGG GTTTCAGCCC AGCAGAAGCA   300

TTATCTTTGC CAGTTGGAGT GCTGGAGACT TTGGATCGGT TGGTGCCACT GAATGGCTAG   360

AGGGATACCT TTCGTCNCCT GCATTTAAAG GCTTTCACTT ATATTAATCT GGATAAAGCG   420

GTTCTTGGTA CCAGCAACTT CAAGGTTTCT GCCAGCCCAC TGTTGTATAC GCTTATTGAG   480

AAAACAATGC AAAATGTGAA GCATCCGGTT ACTGGGCAAT TTCTATATCA GGACAGCAAC   540
```

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 27 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo Sapien
      (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
      (B) CLONE: Prostate Specific Membrane Antigen

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
ACGGAGCAAA ACTTTCAGCT TGCAAAG                                       27
```

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 9 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo Sapien
      (F) TISSUE TYPE: Carcinoma

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```
    (vii) IMMEDIATE SOURCE:
          (B) CLONE: Prostate Membrane Specific Antigen


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

     Thr Glu Gln Asn Phe Gln Leu Ala Lys
     1               5

(2) INFORMATION FOR SEQ ID NO:32:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 36 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (vi) ORIGINAL SOURCE:
          (A) ORGANISM: Homo Sapien
          (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
          (B) CLONE: Prostate Specific Membrane Antigen


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CTCTTCGGCA TCCCAGCTTG CAAACAAAAT TGTTCT                              36

(2) INFORMATION FOR SEQ ID NO:33:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 36 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (vi) ORIGINAL SOURCE:
          (A) ORGANISM: Homo Sapien
          (F) TISSUE TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
          (B) CLONE: Prostate Specific Membrane Antigen


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AGAACAATTT TGTTTGCAAG CTGGGATGCC AAGGAG                              36

(2) INFORMATION FOR SEQ ID NO:34:
```

```
(i)   SEQUENCE CHARACTERISTICS:
      (A)  LENGTH: 12 amino acids
      (B)  TYPE: amino acid
      (C)  STRANDEDNESS: double
      (D)  TOPOLOGY: linear

(ii)  MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv)  ANTI-SENSE: NO

(vi)  ORIGINAL SOURCE:
      (A)  ORGANISM: Homo Sapien
      (G)  CELL TYPE: Carcinoma

(vii) IMMEDIATE SOURCE:
      (B)  CLONE: Prostate Specific Membrane Antigen


(xi)  SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:35:

```
(i)   SEQUENCE CHARACTERISTICS:
      (A)  LENGTH: 6 amino acids
      (B)  TYPE: amino acid
      (C)  STRANDEDNESS: double
      (D)  TOPOLOGY: linear

(ii)  MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv)  ANTI-SENSE: NO

(vi)  ORIGINAL SOURCE:
      (A)  ORGANISM: Homo Sapien
      (G)  CELL TYPE: Carcinoma

(vii) IMMEDIATE SOURCE:
      (B)  CLONE: Prostate Specific Membrane Antigen


(xi)  SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asp Glu Leu Lys Ala Glu
1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

```
(i)   SEQUENCE CHARACTERISTICS:
      (A)  LENGTH: 6 amino acids
      (B)  TYPE: amino acid
      (C)  STRANDEDNESS: double
      (D)  TOPOLOGY: linear

(ii)  MOLECULE TYPE: peptide
```

```
    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (vi) ORIGINAL SOURCE:
           (A) ORGANISM: Homo Sapien
           (G) CELL TYPE: Carcinoma

    (vii) IMMEDIATE SOURCE:
           (B) CLONE: Prostate Specific Membrane Antigen


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

     Asn Glu Asp Gly Asn Glu
     1               5

(2) INFORMATION FOR SEQ ID NO:37:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 6 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
           (A) ORGANISM: Homo Sapien
           (G) CELL TYPE: Carcinoma

     (vii) IMMEDIATE SOURCE:
           (B) CLONE: Prostate Specific Membrane Antigen


      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

     Lys Ser Pro Asp Glu Gly
     1               5

(2) INFORMATION FOR SEQ ID NO:38:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 17 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
           (A) ORGANISM: Homo Sapien
           (G) CELL TYPE: Carcinoma
```

(vii) IMMEDIATE SOURCE:
    (B) CLONE: Prostate Specific Membrane Antigen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Gly Ala Leu Val Leu Ala Gly Gly Phe Phe Leu Leu Gly Phe Leu
1   5   10   15

Phe